PROTEIN SYNTHESIS IN A FISH HEART: RESPONSES TO INCREASED POWER OUTPUT

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Summary

The effects of exercise on the rates of protein synthesis in the chambers of the trout heart were investigated *in vitro* and *in vivo*. An *in vitro* rainbow trout heart preparation was developed which permitted perfusion of the coronary supply to the compact region of the ventricular muscle. This preparation was used to examine the mechanical responses to preload pressures, the oxygen consumption at different power outputs and the rates of protein synthesis in the various heart components. By increasing preload pressure it was possible to double cardiac output, oxygen consumption and power output without changing heart rate. Mechanical efficiency of the hearts was approximately 20%. Perfusion of the coronary vessels improved cardiac output. Protein synthesis was measured in isolated hearts by the incorporation of [^{3}H]phenylalanine added at high concentration (1·35 mmol l^{-1}) to the perfusion medium. The various chambers of the heart showed marked differences in their rates of protein synthesis. Increasing cardiac output and power output *in vitro* by twofold over 20 min increased the fractional rate of protein synthesis by approximately 2·5-fold in the atrium and ventricle but did not affect the rates in the bulbus arteriosus. Perfusion of the coronary vessels significantly increased the rates of protein synthesis of the compact layer of the ventricle. *In vivo* there were no significant differences in the fractional protein synthesis rates between the atrium and ventricle; slow-speed continuous swimming over 40 min (1·5 body lengths s^{-1}) caused an increase in the rates of protein synthesis in all the chambers except the bulbus arteriosus. The stimulation in the fractional rates of protein synthesis by approximately 32% was not as great as *in vitro*. Both *in vivo* and *in vitro* the increased rates of protein synthesis occurred without any change in RNA to protein ratios, indicating an improved activity of protein synthesis per unit of RNA. It is concluded that short-term increases in cardiac contractility, possibly acting through the mechanical stretch on the cardiac muscle, stimulated protein synthesis, particularly in the ventricle, through increased ribosomal activity.

Key words: heart, Starling response, oxygen consumption, protein synthesis, exercise, RNA, trout.

Introduction

Slow-speed continuous swimming stimulates the growth rate of salmonid fish (Davison & Goldspink, 1977; Greer Walker & Emerson, 1978; Nahhas, Jones & Goldspink, 1982; Davie, Wells & Tetens, 1986). Recently the changes in protein turnover that accompany this stimulation in growth have been investigated (Houlihan & Laurent, 1987). In tank-rested, spontaneously active control fish the ventricle retained 18 % of the protein synthesized as growth. The remainder was lost through protein degradation. In fish swimming continuously at a slow speed, (1 body length s^{-1}), both protein synthesis and degradation were stimulated above the control values, but the preponderance of synthesis over degradation resulted in a doubling of the growth rate. As soon as the fish stopped swimming, the rate of protein synthesis in the ventricle fell to that of control fish. The increased growth rate may be accompanied by a relative hypertrophy of the ventricle in exercised fish (Hochachka, 1961) although Houlihan & Laurent (1987) found no increase in the relative ventricle mass in trained rainbow trout.

In mammals the response of the heart to an increased workload is to increase its relative protein mass. The means by which this is brought about *in vivo* are unclear but *in vitro* increased cardiac work causes an elevation in the rate of protein synthesis (Schreiber, Oratz & Rothschild, 1966; Hjalmarson & Isaksson, 1972; Zak & Rabinowitz, 1979; Morgan, Chua, Fuller & Siehl, 1980; Kira, Kochel, Gordon & Morgan, 1984) which occurs without any change in RNA content (Smith & Sugden, 1983*b*). Kira *et al.* (1984) suggest that stretch on the ventricular wall is the mechanical parameter most closely correlated with the increase in protein synthesis. The mammalian heart shows considerable regional variation in protein synthesis rate (Smith & Sugden, 1983*a*), although there are differences between the results from *in vivo* and *in vitro* experiments (Preedy, Smith, Kearney & Sugden, 1985).

The aim of the present study was to investigate the effects of swimming on the rates of protein synthesis in the various chambers of trout hearts. An *in vitro* preparation was used to investigate the effects of increased input pressures on cardiac output, power output, oxygen consumption and protein synthesis. Additionally, *in vitro* experiments were carried out to determine the effects of coronary perfusion on cardiac performance, oxygen consumption and protein synthesis. The *in vivo* experiments compared the rates of protein synthesis in the various regions of the heart in control and active rainbow trout. A speed of 1·5 body length s^{-1} was chosen as this has been found to give high growth rates in trout (Greer-Walker & Emerson, 1978). At this swimming speed the data of Kiceniuk & Jones (1977) suggest that cardiac output increases by a factor of two.

Protein synthesis rates were measured using the incorporation of [^{3}H]phenylalanine in the presence of high levels of phenylalanine, thereby ensuring a high and constant specific radioactivity in the intracellular free pool (Garlick, McNurlan & Preedy, 1980; Smith, Palmer & Reeds, 1983). The RNA content of the fish hearts was measured so that the rate of protein synthesis could be expressed as the RNA

activity (fractional rate of protein synthesis divided by the RNA to protein ratio; Millward *et al.* 1973).

Materials and methods

Animals

Rainbow trout (*Salmo gairdneri* Richardson) were obtained from local fish farms and held in flowing fresh water at Aberdeen University at 10–15°C. They were fed commercial fish food (Ewos-Baker Ltd) four times a day. The mean mass ($\pm$s.e.) of the animals used for the *in vitro* experiments was $715 \cdot 0 \pm 32 \cdot 8$ g ($N = 17$) and for the *in vivo* experiments the mean mass was $300 \cdot 5 \pm 21 \cdot 0$ g ($N = 20$).

In vitro *experiments*

Heart preparation

Fish were anaesthetized with benzocaine ($0 \cdot 05$ g l^{-1}) and injected in the caudal vein with 80 i.u. of heparin in $0 \cdot 5$ ml of saline 2 min before being killed by a sharp blow to the head and transection of the spinal cord. Subsequent procedures were as described by Farrell (1987). Hearts infected with the digenean parasite *Apatemon gracilis* were rejected since performance is impaired (Tort, Watson & Priede, 1987).

The heart was held in a perfusion chamber filled with saline and fitted with two inputs for the sinus venosus/atrium and the coronary artery cannulae (Fig. 1). The perfusion circuit was similar to that used by Farrell (1987) except that the coronary circulation was also perfused from a constant-head reservoir. The perfusate entering the coronary artery drained into the atrium (Farrell, 1987). The perfusion chamber and reservoirs were maintained at 10°C with a temperature-controlled water bath and circulating heater/cooler. Input and output pressures and input pressure to the coronary circulation were measured through saline-filled sidearms connected to a Statham P23 B pressure transducer.

The height of the input and output pressure heads was varied to change the input pressure and diastolic afterload, respectively. Samples for P_{O_2} measurement were taken through saline-filled sidearms (Fig. 1). The perfusate was Cortland saline as modified by Farrell, MacLeod & Chancey (1986).

Protocols

Control conditions

Each heart reached a steady-state condition over 5–15 min. During this period the mean output pressure was 50 cmH$_2$O (1 cmH$_2$O $= 98 \cdot 1$ Pa) and the cardiac output ($\dot{V}$b) was adjusted to approximately 15 ml min^{-1} kg^{-1} by changing the input pressure between $0 \cdot 6$ and 3 cmH$_2$O as necessary. In intact trout resting $\dot{V}$b is $17 \cdot 6$ ml kg^{-1} min^{-1} with venous and ventral aortic blood pressures of 2 cmH$_2$O and 47–54 cmH$_2$O, respectively (Kiceniuk & Jones, 1977). The heart rate was determined by the intrinsic rhythm of the pacemaker and was found to vary among
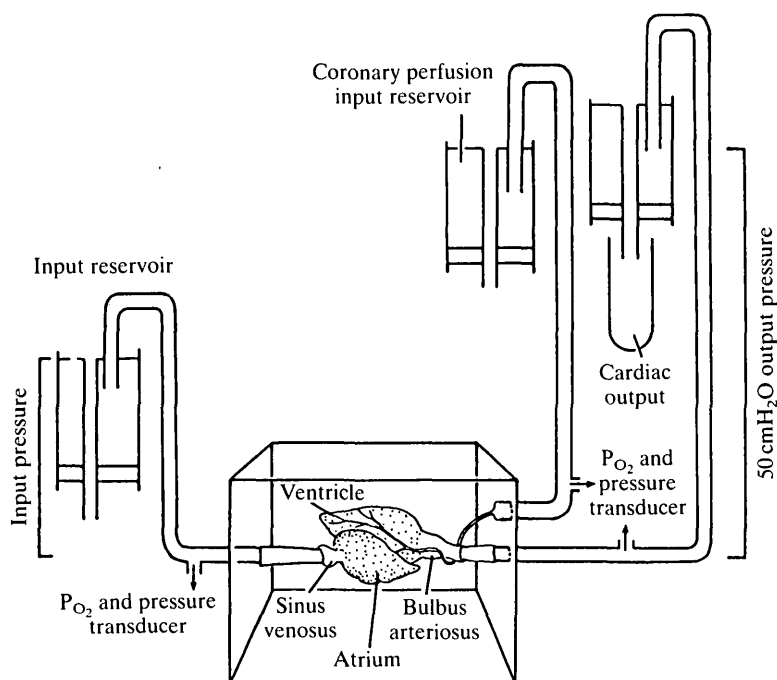
Fig. 1. Diagram of the apparatus used for the heart perfusion studies. The atrium received perfusion fluid with an input pressure controlled by the height of the input reservoir. The cardiac output was collected from the cannulated bulbus arteriosus and the output pressure was controlled by the height of the output reservoir. The coronary artery was cannulated and received perfusion fluid from the coronary input reservoir. Samples of perfusate and cardiac output were collected for measurements of P_{O_2}.

preparations. The coronary input pressure was adjusted to obtain a flow of about 2 % of the $\dot{V}b$ in accordance with Farrell & Graham (1986).

Group 1: time course

The performance of hearts without coronary circulation was monitored over 30 min. Input pressures were kept between 0·6 and 3 cmH_2O. Output pressure was 50 cmH_2O.

Group 2: Starling response (volume loading)

From control conditions, the input pressure was increased to obtain a $\dot{V}b$ of 21 ml min^{-1} kg^{-1} and then further increased so as to double the initial power output. Diastolic end pressure was constant. Experiments were carried out with and without coronary circulation.

Group 3: oxygen consumption

Oxygen consumption of the hearts was determined from the difference in P_{O_2} between the input perfusate and the cardiac output. Six hearts were used and there

was an alternation in the starting condition of experiments for successive hearts: to commence with or without coronary perfusion. Starting with a low input pressure and no coronary perfusion the input and output P_{O_2} values were measured in discrete samples taken anaerobically through the appropriate sidearms. After two successive samples had given the same values the input pressure was raised to double the initial power output. Input and cardiac output P_{O_2} values were again sampled until steady-state values were obtained. The input pressure was then returned to a low value and the experiment repeated with coronary perfusion. Each experiment lasted approximately 40 min. There was no significant difference between the results obtained from experiments commencing or ending with coronary perfusion.

The hearts received aerated saline (mean P_{O_2} 149·0 ± 2·3 mmHg; 1 mmHg = 133·3 Pa) at 10°C through the input and, where it occurred, through the coronary perfusion at all times.

In all experiments the perfusion chamber was filled initially with aerated saline, sealed and stirred throughout with a rotating magnetic bar. To determine the extent of oxygen consumption by the heart from the chamber, samples of chamber fluid were taken and their P_{O_2} values measured. Also, samples of saline were taken from a filled chamber without a heart to determine the extent of any oxygen uptake occurring from the saline. There were no significant differences in the results obtained from these two procedures and it was concluded that there was no significant oxygen consumption by the heart from the bathing saline.

Group 4: protein synthesis

Protein synthesis was measured in the presence of a high concentration of phenylalanine (Garlick *et al.* 1980; Smith *et al.* 1983). The labelled medium used for cardiac and coronary perfusion contained 1·35 mmol l^{-1} phenylalanine with a nominal specific activity of 1600 disints min^{-1} nmol^{-1}. This was prepared by adding 1 ml of a stock solution containing 135 mmol l^{-1} phenylalanine and L-[2,6-^{3}H]phenylalanine at a concentration of 100 μCi ml^{-1} (3·7 MBq ml^{-1}) to 100 ml of the perfusion medium described above. The hearts, either in 'rest' conditions (with or without coronary circulation) or at high work levels, were first given 10 min in the normal medium to achieve stable performance before the phenylalanine was added. At the end of the incubation, which lasted for 3–30 min in time-course experiments, the hearts were removed from the labelled medium and immediately divided into the atrium, the base of the ventricle (at the junction of the atrium and ventricle), the ventricular apex, the spongy layer just behind the apex of the ventricle, and the bulbus arteriosus. It was not possible to take a sufficiently large sample of the pure compact ventricular layer. The dissections took place on ice and lasted 2 min. The spongy sample was taken by scraping away the spongy tissue from the compact tissue with a sharp scalpel. The heart fragments were then frozen in liquid nitrogen and stored at −30°C.

Tissue treatment to measure the specific radioactivity in the free pool and protein was similar to that described by Houlihan, McMillan & Laurent (1986).

The RNA content of the tissues was determined by the orcinol method (Mejbaum, 1939).

In vivo *protein synthesis*

Rainbow trout were divided into non-swimming and swimming groups. The non-swimming animals were taken from their holding tanks and injected in the caudal vein, without previous anaesthesia, with a solution of $140 \, \text{mmol} \, \text{l}^{-1}$ phenylalanine in trout saline, pH 7·9. This solution contained L-$[2,6-^3\text{H}]$phenylalanine at $67 \, \mu\text{Ci} \, \text{ml}^{-1}$ ($2.4 \, \text{MBq} \, \text{ml}^{-1}$). The injection dose was $1 \, \text{ml} \, 100 \, \text{g}$ fresh mass^{-1}. After the injection the fish were placed individually in aerated fresh water at 13°C where they recovered immediately and rested quietly. The animals were killed 40 min after the injection.

The swimming animals were injected with the same dose and then placed in a Perspex chamber which formed part of an annular flume (Priede, 1974). When the animals had recovered (usually after 2 min) the water flow was gradually increased over 1 min, by adjusting a water pump, until it was approximately 1·25–1·5 body lengths s^{-1}. The fish swam well for 40–60 min, after which they were killed with a sharp blow to the head followed by pithing and destruction of the brain. Water temperature was 13°C and the fish were swum in pairs.

The animals in both groups were dissected on ice immediately after death, and the various chambers of the heart removed and frozen in liquid nitrogen. Tissue treatment for the measurement of the protein-bound and free phenylalanine and RNA to protein ratios was as described above for the *in vitro* hearts.

Morphology

Transverse cryostat sections of the ventricle were cut, stained with haematoxylin and eosin and the areas of the compact and spongy myocardium determined from *camera lucida* projections as described by Santer & Greer Walker (1980). Sections from three levels of the ventricle were examined and the mean ratio of spongy to compact muscle was determined.

Measurement and calculations

Cardiac output was collected over 30 s, weighed, corrected for temperature and salinity and expressed as a volume measurement. The heart rate was obtained from the pressure records. Stroke volume was calculated as $\text{Vs} = \dot{\text{V}}\text{b}$ ($\text{ml} \, \text{min}^{-1}$)/$f_\text{H}$ (beats min^{-1}). Power output (mW) and mechanical efficiency of the heart were calculated as described by Farrell, Wood, Hart & Driedzic (1985). Pressures were referenced to the level of saline in the chamber. Corrections were made for the pressure drops across the cannulae. $\dot{\text{V}}\text{b}$ and Vs were calculated per kilogram of total fish body mass (wet mass measured before the experiment) and the power output was calculated per gram ventricular mass (blotted wet mass determined at the end of the experiment).

The coronary flow was calculated from the fall in pressure through the input tube corrected for coronary cannula resistance with respect to zero flow conditions.

The oxygen partial pressure (P_{O_2}) of samples was determined with a Radiometer oxygen electrode thermostatted at 10 °C. The electrode was calibrated with air and zero solution.

Oxygen consumption was calculated from:

$$\dot{V}_{O_2} = (Pi_{O_2} - PVb_{O_2}) \times \alpha wO_2 \times \dot{V}b \,,$$

where Pi_{O_2} is the input (perfusate) P_{O_2} and PVb_{O_2} is the P_{O_2} of the cardiac output. αwO_2 is the solubility coefficient for the saline and $\dot{V}b$ is the cardiac output (ml g ventricle^{-1} min^{-1}). All gas volume measurements were corrected to STPD.

Utilization of oxygen during the passage of the perfusion fluid through the heart was calculated as:

$$\% \text{ utilization} = \frac{Pi_{O_2} - PVb_{O_2} \times 100}{Pi_{O_2}} \,.$$

Protein synthesis (k_s, % day^{-1}, the fraction of the total protein synthesized per day) was calculated as:

$$k_s = \frac{S_b}{S_a} \times \frac{1440}{t} \times 100 \,,$$

where S_b is the specific radioactivity of protein-bound phenylalanine (disints min^{-1} nmol^{-1}), S_a is the specific radioactivity of the free-pool phenylalanine (disints min^{-1} nmol^{-1}) and t is the incubation time (min). The protein contents of the tissues are expressed as mg protein g^{-1} fresh mass of heart tissue and the RNA to protein ratio as μg RNA mg protein^{-1}. The total amount of protein synthesized was calculated by multiplying the fractional synthesis rate by the total amount of protein in the tissue under consideration.

The RNA activity was calculated as the ratio of fractional synthesis rate to RNA to protein ratio (g protein synthesized g RNA^{-1} day^{-1}) (Millward *et al.* 1973).

Statistically significant differences ($P < 0.05$) were determined using Student's *t*-test.

Results

In vitro *heart performance*

Over a 30-min period there were no significant changes in any of the parameters in low-work hearts without coronary perfusion. The cardiac output was similar to that of intact resting trout (17·6 ml kg^{-1} min^{-1}; Kiceniuk & Jones, 1977) and to the control conditions of an *in situ* trout heart (Farrell *et al.* 1986). However, the input pressures used here were higher than those used by Farrell *et al.* (1986). Heart rate and power output were also lower than those found by Farrell *et al.* (1986). The

mean output pressures used here are similar to those found in resting trout (47–54 cmH$_2$O, Kiceniuk & Jones, 1977).

In hearts without coronary circulation increasing input pressure produced an increase in cardiac output and power output with no significant change in heart rate (Fig. 2). Thus, the changes in cardiac output were attributable to changes in stroke volume. Coronary perfusion improved the heart's performance such that, at the same low or high input pressures, hearts with coronary perfusion had significantly higher cardiac outputs than hearts without coronary perfusion (Fig. 2). As coronary flow amounted to only 2% of the cardiac output the supplementation of the latter by the former was not sufficient to account for the
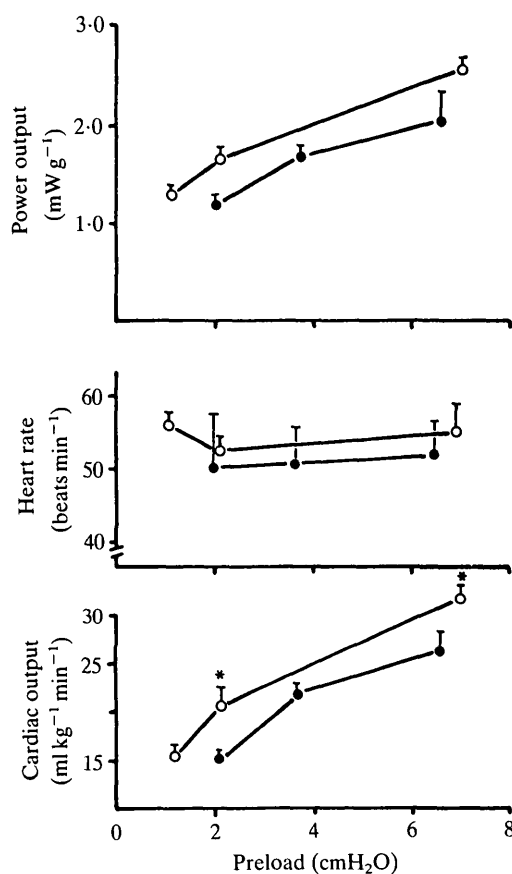

Fig. 2. The effects of increasing preload on cardiac performance of *in vitro* hearts with (O) or without (●) coronary perfusion. Afterloads were 50 cmH$_2$O throughout. The temperature was 10°C. Mean masses of fish and ventricles for the experiments without coronary circulation were 707·8 ± 59·15 g and 0·67 ± 0·05 g, respectively, $N = 4$; for the experiments with coronary circulation they were 766·0 ± 27·3 g and 0·63 ± 0·015 g, respectively, $N = 3$. * $P < 0.05$ for comparisons made at the same input pressures between values made with and without coronary perfusion.

greater cardiac output. There were no significant differences in the heart rate between hearts with or without coronary perfusion. Equivalence of calculated power output was also achieved at lower input pressures in the hearts with coronary perfusion. However, there were no significant differences in power output between hearts with or without coronary perfusion when they were compared at the same input pressures. In hearts both with and without coronary perfusion it was particularly noticeable that the cardiac output was more sensitive to input pressures at the lower values. This has also been reported by Farrell *et al.* (1986) for hearts without coronary perfusion.

As there was no detectable oxygen consumption from the saline bathing the heart (see Materials and methods), oxygen extraction from the perfusate was the sole route of oxygen consumption in the hearts without coronary perfusion. With a mean P_{O_2} of 149 mmHg in the perfusion fluid there was $18 \cdot 3 \pm 1 \cdot 8 \%$ extraction of the oxygen at low input pressure, giving an oxygen consumption of $19 \cdot 9 \pm 2 \cdot 2 \, \mu l \, O_2 g \, \text{ventricle}^{-1} \, \text{min}^{-1}$ (Fig. 3). There was no significant difference
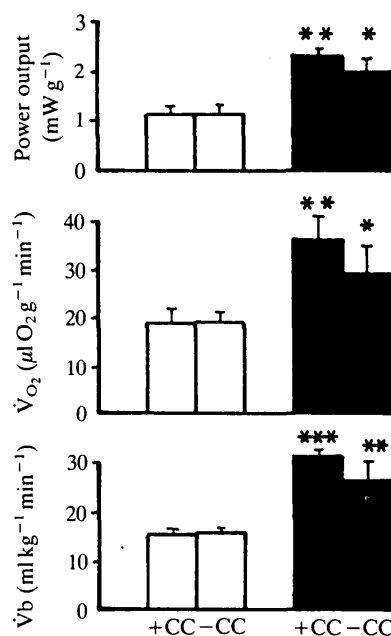

Fig. 3. Mean ($\pm$s.e.) cardiac variables (power output, $mW \, g \, \text{ventricle}^{-1}$; $\dot{V}_{O_2}$, oxygen consumption, $\mu l \, O_2 \, g \, \text{ventricle}^{-1} \, \text{min}^{-1}$; $\dot{V}b$, cardiac output, $ml \, kg \, \text{fish mass}^{-1} \, \text{min}^{-1}$) for isolated hearts with (+CC) and without coronary perfusion (−CC) subjected to volume loading. Input pressures (cmH_2O) were $1 \cdot 1 \pm 0 \cdot 21$ (+CC) and $1 \cdot 9 \pm 0 \cdot 32$ (−CC) in low-work conditions (unshaded bars) and $6 \cdot 9 \pm 1 \cdot 0$ (+CC) and $7 \cdot 2 \pm 1 \cdot 62$ (−CC) in high-work conditions (shaded bars). Afterloads were $50 \, cmH_2O$ throughout. The temperature was $10°C$. The mean fish and ventricle masses were $646 \cdot 3 \pm 9 \cdot 8 \, g$ and $0 \cdot 63 \pm 0 \cdot 04 \, g$, respectively, $N = 5$. $*P < 0 \cdot 05$, $**P < 0 \cdot 01$, $***P < 0 \cdot 001$ for comparisons between low-work and high-work conditions.

in the oxygen extraction or consumption between hearts supplied with or denied coronary perfusion at the same cardiac output. In these experiments the presence of coronary perfusion did not significantly lower the input pressure necessary to generate a cardiac output of $15 \, ml \, kg^{-1} \, min^{-1}$.

Increasing the input pressure resulted in an increase in cardiac output, power output and oxygen consumption (Fig. 3). A doubling of cardiac output in hearts with coronary perfusion led to a doubling of the oxygen consumption and power output. There were no significant differences in the extraction of oxygen from the perfusate by hearts without and with coronary flow at the higher input pressure (mean $18 \cdot 0 \pm 3 \cdot 8 \%$ and $16 \cdot 0 \pm 1 \cdot 5 \%$, respectively) and, therefore, the increased oxygen consumption was due to the increased flow (cardiac output). Under high-work conditions there were no significant differences in any of the parameters between hearts receiving or denied coronary perfusion.

There was a linear relationship between the oxygen consumption of the hearts and the cardiac output and power output. Regression analysis of power output (x, $mW \, g \, ventricle^{-1}$) and oxygen consumption (y, $\mu l O_2 \, g \, ventricle^{-1} \, min^{-1}$) gave: $y = 3 \cdot 067 + 12 \cdot 67x$, $N = 11$, $r = 0 \cdot 91$, $P < 0 \cdot 001$. Mechanical efficiency of the heart in low-work conditions ($20 \cdot 07 \pm 2 \cdot 34 \%$) did not differ significantly from that in high-work conditions ($21 \cdot 11 \pm 3 \cdot 67 \%$).

These experiments show that the *in vitro* hearts can generate resting physiological cardiac outputs at physiological input pressures and that the hearts respond to increased input pressures by increasing power output by up to a factor of two. Perfusion of the coronary vessels in the compact layer of the heart lowered the input pressures necessary to achieve physiological cardiac outputs and improved the cardiac output when comparisons were made at the same input pressures. However, perfusion of the coronary vessels did not change oxygen consumption or power output significantly when comparisons were made at the same input pressures.

Protein synthesis in vitro

Equilibration of phenylalanine in the homogenate pool and rate of incorporation of labelled phenylalanine into heart protein

The concentration of phenylalanine in the incubation medium, $1 \cdot 35 \, mmol \, l^{-1}$, was considerably higher than the normal intracellular (homogenate) concentration of $90 – 220 \, nmol \, g \, fresh \, mass^{-1}$ (Haschemeyer & Smith, 1979). Under incubation conditions of low work without coronary circulation the phenylalanine concentration increased very rapidly. Within 3 min it had risen by at least 2- to 4-fold and it remained stable for at least 18–30 min, although the whole ventricle samples and the bulbus arteriosus had significantly lower phenylalanine concentrations than the atrium and the spongy layer of the ventricle. The specific radioactivity of the homogenate pool of free phenylalanine attained close to 100 % of the medium value after 3 min and was maintained at that value at 15 and 30 min (Fig. 4A).
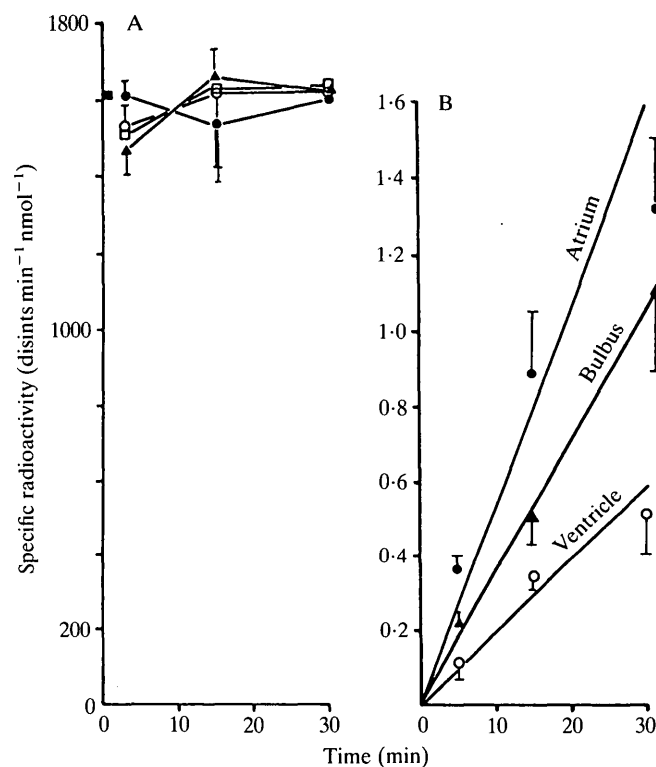
Fig. 4. (A) Specific radioactivity of free-pool phenylalanine of three chambers of the heart and the spongy layer of the ventricle after [^{3}H]phenylalanine had been introduced into the perfusion fluid. Specific radioactivity of the perfusion fluid is shown on the ordinate (■). (B) Incorporation of labelled phenylalanine into proteins following the introduction of the [^{3}H]phenylalanine into the perfusion fluid of *in vitro* hearts. The hearts ($N = 7$) were in low-work conditions without coronary circulation. The temperature was 10°C. The mean mass of the animals used was 702·2 ± 49·9 g. (●) atrium, (○) ventricle, (□) spongy layer of the ventricle, (▲) bulbus arteriosus.

The mean specific activity of the protein-bound phenylalanine increased linearly during the course of the incubation in the bulbus arteriosus (Fig. 4B). There was some indication of a decreased rate of incorporation in the atrium and ventricle after 30 min. All subsequent results on the fractional rates of protein synthesis *in vitro* were taken from incubations which had a mean duration of 21·2 ± 1·5 min.

In vitro *protein synthesis rates*

There were significant differences in the rates of [^{3}H]phenylalanine incorporation into proteins among the various chambers of the heart (Figs 4, 5). In resting/control hearts without coronary circulation the sequence of fractional rates of protein synthesis rates in the various heart components was: atrium = spongy layer = bulbus arteriosus > whole ventricle ($P < 0.05$).
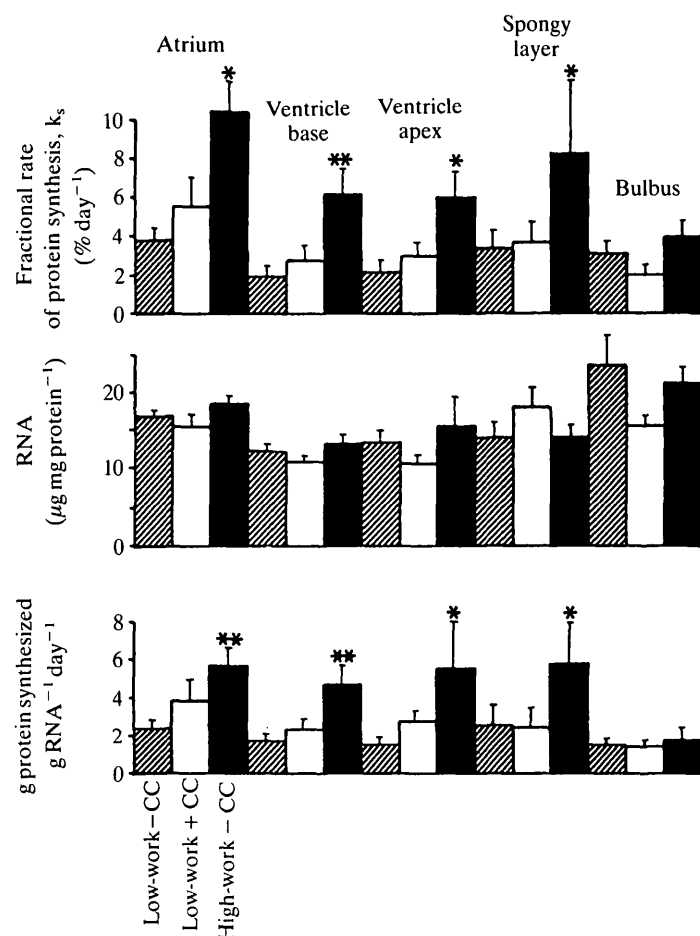
Fig. 5. Fractional rates of protein synthesis, RNA to protein ratios and the amount of protein synthesized per gram of RNA per day for *in vitro* hearts. The results are from control-work hearts with (bars, $N = 7$) and without (cross-hatched bars, $N = 4$) coronary circulation and for high-work hearts without coronary circulation (filled bars, $N = 6$). The conditions of the hearts are described in Table 1. $* P < 0.05$, $** P < 0.01$ for comparisons between the low-work hearts without coronary perfusion and the high-work hearts also without coronary perfusion.

In low-work hearts without coronary perfusion the RNA to protein ratios of the chambers were significantly different with the following ranking: bulbus arteriosus > atrium > whole ventricle = spongy layer. From the calculation of the RNA activity ($k_s RNA$; g protein g $RNA^{-1} day^{-1}$) the atrium and the spongy layer of the ventricle have the highest values (Fig. 5).

Perfusing the coronary circulation at low-work conditions although giving a significantly higher cardiac output at lower preloads (Table 1) resulted in no significant changes in fractional synthesis rates when compared with low-work conditions (Fig. 5). There were also no significant differences in protein to fresh-

mass ratios (results not shown), RNA to protein ratios or RNA activity between the low-work hearts with and without coronary circulation.

Increasing cardiac output and power output by a factor of 1·8 and 2·1, respectively, through increased preload pressures (Table 1) stimulated the fractional rate of protein synthesis in the atrium and the ventricle by 2·7-fold (Fig. 5). The spongy layer of the ventricle showed a 2·5-fold increase. The bulbus arteriosus showed no significant change in the fractional rate of protein synthesis with increased power output. The ranking of the tissues in terms of fractional synthesis rates now becomes: atrium = spongy layer > ventricle > bulbus arteriosus.

There were no significant differences in either protein to fresh-mass ratios in the various tissue samples between the high-work and low-work groups (results not shown) or in RNA to protein ratios (Fig. 5). Therefore the RNA activity increased in all the tissues except the bulbus arteriosus when the hearts were working at high power output (Fig. 5).

Calculation of the total amount of protein that was synthesized in the various heart components shows that in low-work hearts without coronary circulation, the ventricle was the chamber with the highest rate of protein synthesis (Table 2). This was due to its size, since it has the lowest fractional rate of protein synthesis. The total amount of protein synthesized in the ventricular spongy layer was calculated from the proportion of the ventricle which it occupied, its protein to fresh-mass ratio and its fractional synthesis rate. The mean ratio of spongy to compact myocardium taken from tracings of transverse sections at three levels of the heart was 2.9:1. Using this value and the measurements from each heart, the spongy layer was found to be the major site of protein synthesis in low-work hearts lacking coronary perfusion (Table 2).

The fractional protein synthesis rate in the compact layer was calculated from the differences between the protein synthesized in the total ventricle and that in the spongy layer; this gave a value of $0·37 \pm 0·18 \%$ day^{-1}, which is significantly lower than that found in the spongy layer ($3·27 \pm 1·09 \%$ day^{-1}, $P < 0·05$) and in the whole ventricle ($2·09 \pm 0·48 \%$ day^{-1}, $P < 0·05$).

In low-work hearts with coronary perfusion the spongy layer accounted for $73·0 \pm 11·4 \%$ of the total, an insignificant difference from the above value. In the presence of coronary perfusion the fractional rate of protein synthesis in the compact layer had increased to $1·94 \pm 0·38 \%$ day^{-1}, significantly different from the value in low-work hearts without coronary circulation ($P < 0·05$, see above).

In high-work conditions the total amounts of protein synthesized increased by approximately 1·6-fold in the atrium and 2·5-fold in the ventricle and ventricular spongy layer. There was no significant change in the bulbus arteriosus (Table 2). The spongy layer again accounted for the majority of the total ventricular protein synthesis. Calculation of the fractional protein synthesis rate in the compact layer gave a value of $2·09 \pm 1·4 \%$ day^{-1} for high-work conditions, a significantly higher value than that obtained in the compact layer from low-work hearts (see above, $< 0·05$). Data were not available to calculate the fractional rate of protein synthesis in the perfused compact layer under high-work conditions.

Table 1. *Cardiac variables during the* in vitro *measurement of protein synthesis*

	Fish mass (g)	Ventricular mass (g)	Input pressure (cmH_2O)	Heart rate (beats min^{-1})	$\dot{V}b$ (ml min^{-1} kg^{-1})	Power (mW g^{-1})
Low-work without CC N=7	687·2 ± 46·1	0·67 ± 0·04	2·4 ± 0·3	47·3 ± 2·7	14·9 ± 0·5	1·2 ± 0·1
Low-work with CC N=4	585·0 ± 22·5	0·63 ± 0·04	1·7 ± 0·32	54·2 ± 1·7	16·2 ± 1·4	1·30 ± 0·12
High-work without CC N=6	661·7 ± 37·0	0·64 ± 0·03	6·4 ± 0·3	45·3 ± 5·4	26·5 ± 1·4	2·5 ± 0·1

Values are means ± S.E.

The hearts were in rest conditions with or without coronary perfusion in low-input pressure conditions and without coronary perfusion in high-input conditions. CC, coronary circulation.

The afterload was always 50 cmH$_2$O.

The temperature was 10°C and the mean duration of the experiments was 21·21 ± 1·5 min.

Table 2. *Rates of protein synthesis (mg protein region^{-1} day^{-1}) of isolated and* in vivo *hearts*

	In vitro		In vivo	
	Low-work	High-work	Controls	Swimming
Atrium	0·42 ± 0·05	0·69 ± 0·18*	0·15 ± 0·06	0·20 ± 0·08*
Ventricle	1·55 ± 0·56	3·99 ± 1·76**	1·05 ± 0·25	1·54 ± 0·61*
Spongy layer of ventricle	1·43 ± 1·07	3·51 ± 0·86**	0·69 ± 0·07	0·90 ± 0·05*
Spongy/total ventricle (%)	92·8 ± 5·6	87·7 ± 8·1	65·0 ± 3·4	58·4 ± 0·90*
Bulbus arteriosus	0·55 ± 0·16	0·72 ± 0·33	0·17 ± 0·08	0·22 ± 0·09

* $P < 0.05$, ** $P < 0.01$ for comparisons between low- and high-work hearts *in vitro* and between resting and swimming fish *in vivo*.

The data for the *in vitro* hearts comes from the low-work and high-work conditions without coronary perfusion.

For each heart the fractional rate of protein synthesis was multiplied by the protein content of the chamber.

Other conditions as in Figs 5 and 6.

Note that the animals used in the *in vivo* experiments were smaller than those used in the *in vitro* experiments.

In vivo *protein synthesis*

In contrast to the *in vitro* results, *in vivo* the ranking of the fractional rates of protein synthesis of the chambers of the heart was: bulbus arteriosus > atrium = ventricle = ventricular spongy layer (Fig. 6). The bulbus arteriosus had significantly higher rates of synthesis compared with all the other regions. There were significant increases in the fractional rates of protein synthesis in all the regions of the heart, except the bulbus, when the animals were made to swim during the protein synthesis determination. This increase amounted to 23 % in the atrium, 40 % in the ventricle and 28 % in the spongy lining of the ventricle. There were no significant differences in the fractional rates of protein synthesis between the various regions of the heart in swimming fish.

There were significant differences between the various regions of the heart in RNA to protein ratios, with the bulbus having the highest values (Fig. 6). There
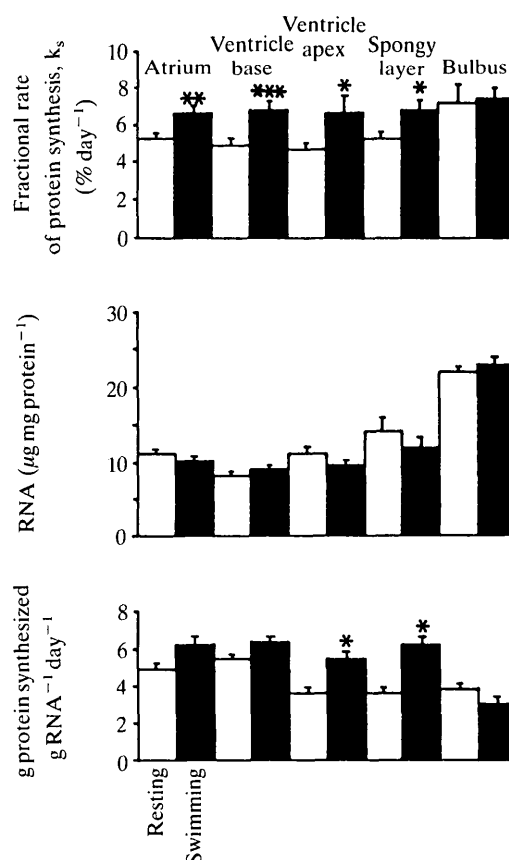

Fig. 6. Fractional rates of protein synthesis, RNA to protein ratios and the amount of protein synthesized per gram of RNA per day for *in vivo* hearts. The results are from control fish resting during the course of the determination (mean ± s.E. mass of the fish 292·8 ± 28·2 g, $N = 11$) and for swimming fish (310·0 ± 32·68 g, $N = 9$). $*P < 0·05$, $**P < 0·02$, $***P < 0·001$ for comparisons between resting and swimming fish.

were no significant differences in RNA to protein ratios between the regions of the hearts of swimmers and non-swimmers. Calculation of the RNA activity shows that this had increased significantly in the swimming fish in the apex of the ventricle and in the spongy layer (Fig. 6).

Calculation of the total amounts of protein synthesized in the various regions of the heart revealed that, as *in vitro*, the ventricle had the highest rates of protein synthesis and that this increased significantly in the swimming fish (Table 2). In resting animals the spongy layer of the ventricle is the major site of protein synthesis but the percentage contribution of the spongy layer to the total ventricular synthesis is significantly less than the value found *in vitro* ($P < 0.05$). In swimming animals the spongy layer makes an even smaller contribution to the total.

Calculation of the fractional rate of protein synthesis in the compact layer of the ventricle gave a mean value of $4.36 \pm 0.31 \%$ day^{-1} in resting fish, which is slightly lower than the value for the whole ventricle of the resting fish ($4.97 \pm 0.26 \%$ day^{-1}). The calculated fractional synthesis rate in the compact layer had risen to $6.80 \pm 0.98 \%$ day^{-1} in the swimming fish, a significant increase over the value in resting fish ($P < 0.05$); in swimming fish the fractional synthesis rates in the compact and spongy layers of the ventricle were not significantly different. Overall the atrium and ventricle showed a 32 % increase in the fractional rate of protein synthesis during swimming, which contrasts with the 155 % increase showed by these chambers in the *in vitro* experiments.

Discussion

In vitro *heart performance*

The *in situ* perfused heart described by Farrell *et al.* (1986) had a superior performance to the isolated heart described here in terms of the input pressure necessary to generate resting $\dot{V}$b, power output and heart rate. The differences in performance between the two preparations are probably attributable to the greater degree of mechanical manipulation necessary for the *in vitro* preparation and the maintenance of the sinus venosus/atrial contact in the *in situ* preparation as carried out by Farrell (1987). However, cannulation of the coronary vessels was not possible *in situ* and this led to the use of the isolated hearts to enable investigations of the effects of coronary perfusion on heart performance. We did not attempt to generate maximal sustained performance with the *in vitro* hearts; the high-work hearts in this study had cardiac outputs of half the maximal value found by Farrell *et al.* (1986) (60 ml min^{-1} kg^{-1}) and estimates of maximal cardiac output in swimming trout (53 ml kg^{-1} min^{-1}; Kiceniuk & Jones, 1977).

Despite the differences between this *in vitro* heart and *in situ* perfused hearts, our preparation performed very well compared with other *in vitro* trout heart preparations (Jensen, 1969; Tort *et al.* 1987). The resting cardiac output was achieved at a physiological input pressure. However, the Starling response

occurred at pressures above those reported for trout swimming at their critical velocity (Kiceniuk & Jones, 1977; Farrell, 1985).

Although this and other studies using perfused hearts have shown the importance of input pressure in determining stroke volume (Farrell, 1985), it is not clear that venous pressure is an important *in vivo* determinant of stroke volume: only insignificant increases in blood pressure were found in the right common cardinal vein of rainbow trout swimming at maximum velocity (Kiceniuk & Jones, 1977). For this reason, it is not safe to conclude that increased input pressures *in vitro* are directly comparable to the factors involved in bringing about increases in cardiac output in swimming fish.

The action of input pressures in increasing cardiac output *in vitro* needs clarification. It has recently been argued by Johansen & Gesser (1986) that the ventricular end-diastolic volume, and hence the subsequent ventricular stroke volume, is dependent on the extent of the atrial contraction. Thus the effects of input pressure on ventricular performance are acting on atrial stroke volume which is the sole agent for ventricular filling. This is particularly important in the control of cardiac output in fish, as data from radiocardiography suggest that in resting fish the ventricle empties completely leaving no residual volume (Johansen & Gesser, 1986).

It was noticeable during the *in vitro* experiments that the atrium expanded considerably before its contraction filled the ventricle. Atrial overdistension was also noted by Farrell (1987) in *in vitro* hearts. This may be an artefact of the *in vitro* heart since the pericardium and surrounding tissue which constrain the maximum volume of the heart *in vivo* were absent *in vitro*. As atrial diastole and filling is the longest event in the cardiac cycle (Johansen & Gesser, 1986), the prolonged exposure of the atrium to the input pressure *in vitro* may have resulted in the high rates of protein synthesis in that chamber, even in low-work hearts, which were not evident *in vivo* (Figs 5, 6).

The *in vivo* and *in vitro* experiments have, however, one factor in common; increases in cardiac output are accompanied by little or no increase in heart rate (Jones & Randall, 1978; Farrell, 1984; Butler, 1986).

The values for myocardial oxygen uptake reported here are similar to those reported previously (Farrell *et al.* 1985; Farrell & Milligan, 1986). The mechanical efficiency of sea raven hearts (Farrell *et al.* 1985) and rainbow trout hearts (Farrell & Milligan, 1986) has been found to be about 15 %. The rainbow trout heart in the present study had an efficiency of about 20%. Fish hearts show a linear relationship between power output and oxygen consumption in volume-loaded conditions. The presence of coronary perfusion in the present experiments did not significantly increase oxygen consumption. This suggests that diffusion of oxygen is not a significant problem in *in vitro* hearts.

This is the first reported study which used the flooding dose method (Garlick *et al.* 1980) for the *in vitro* measurement of protein synthesis rates of working fish muscle. The *in vitro* validation experiments indicated that the flooding was very rapid and that the free-pool specific radioactivity remained constant during the

course of the incubation. Loughna & Goldspink (1985) have shown that the elevated levels of phenylalanine do not themselves stimulate the rates of protein synthesis. Validation of the free-pool flooding technique for *in vivo* studies of rainbow trout comes from previous reports (Houlihan *et al.* 1986; Houlihan & Laurent, 1987).

The simultaneous measurements of protein synthesis and oxygen consumption in the isolated hearts allow an estimation of the proportion of the total energy expenditure used on protein synthesis. Using the values for the total protein synthesis rates of hearts *in vitro* in low-work conditions from Table 2 and a value of 50 mmol ATP g protein^{-1} (Reeds, Fuller & Nicholson, 1985), the energy cost of protein synthesis can be calculated to have accounted for 1·9 % of the total aerobic energy consumption of the heart. In high-work conditions without coronary circulation protein synthesis accounted for 2·6 % of the total aerobic consumption. These values are in sharp contrast to recent values for whole fish. Houlihan, Hall, Gray & Noble (1988) have calculated for cod that the energy cost of protein synthesis in starving fish represents 24 % of the total oxygen consumption and that this value rises to 42 % in feeding fish. Clearly in muscle tissues such as the heart, protein synthesis is a relatively small component of the energy demand compared with the amount of energy used for muscle contraction.

All the *in vitro* values for fractional protein synthesis rates of low-work hearts with coronary circulation are lower than the *in vivo* values. Direct comparisons between the two groups of animals are complicated by the greater mass of the fish used for *in vitro* experiments, there being a marked decline in ventricular fractional protein synthesis rate with increasing body size (Houlihan *et al.* 1986). However, using scaling values given by Houlihan *et al.* (1986) for rainbow trout, the whole ventricle values from the *in vitro* experiments were converted to those of a 300-g animal (the mean *in vivo* resting animal mass), giving a mean fractional synthesis rate of 3·2 % day^{-1} compared with the measured value of 4·8 % day^{-1} *in vivo*. From this we may conclude that the resting *in vitro* heart was synthesizing proteins at approximately 67 % of its possible *in vivo* resting rate. This is an extremely good performance for an *in vitro* preparation since isolation of muscle often results in reduced fractional protein synthesis rates (Palmer, Reeds, Lobley & Smith, 1981; Goldspink, Garlick & McNurlan, 1983). *In vitro* rat hearts exhibit fractional protein synthesis rates for the atrium and ventricle of approximately 60 % of *in vivo* rates (Preedy, Smith, Kearney & Sugden, 1984; Preedy *et al.* 1985).

The protein synthesis rates in teleost fish are markedly different from those in mammals. In rats the combined atria and ventricles growing at 1·4 % day^{-1} in a 211-g animal, have fractional synthesis rates of 12·0 % day^{-1} and an RNA activity of 11·5 g protein g RNA^{-1}day^{-1} (Lewis, Kelly & Goldspink, 1984). In the present experiments the trout ventricle growing at 0·24 % day^{-1} (Houlihan *et al.* 1986b) synthesized protein at 5 % day^{-1} with an RNA activity of 4 g protein g RNA^{-1} day^{-1}. This indicates that the rat ventricle retains a greater proportion of the synthesized protein as growth and that in trout the RNA is far less active in producing protein. However, the temperature difference between rats and trout

can more than account for the differences in RNA activity. From the above results the Q_{10} for the RNA activity is 1·5 and Haschemeyer (1978) has suggested a Q_{10} of 2·7 for protein synthesis rates.

Protein synthesis in response to exercise: in vitro *and* in vivo

The important results obtained from this study are the stimulation of fractional rates of protein synthesis in the atrium and ventricle with short-term 'exercise' both *in vitro* and *in vivo*. They confirm that swimming stimulates the rate of ventricular protein synthesis (Houlihan & Laurent, 1987), which may give rise to increased growth rates. However, in that study, fish trained for 6 weeks had fractional rates of protein synthesis in the ventricle which were double those of the control fish when the trained animals were swimming at 1 body length s^{-1} during the course of the protein synthesis measurement. Although the animals used were smaller than those in the present study (91 g compared with 300 g), the greater stimulation of protein synthesis that followed training compared with the immediate effects of swimming on previously untrained animals reported here may indicate longer-term effects of continuous swimming, such as increases in RNA to protein ratios in the tissues.

The present results also agree with experiments on mammals showing the sensitivity of protein synthesis in the heart to increased work load. In the perfused rat heart, increases in filling pressure with an afterload held constant increase rates of protein synthesis in the atrium by 30–40 % (Smith & Sugden, 1983b). However, acute volume overloading *in vitro* does not stimulate ventricular protein synthesis in mammals although increased afterload does (Schreiber *et al.* 1966; Schreiber, Evans, Oratz & Rothschild, 1981; Smith & Sugden, 1983b; Kira *et al.* 1984).

The results from the trout heart present a more consistent effect of increased volume loading on the rate of protein synthesis. Fractional rates of protein synthesis increase by approximately 2·5-fold in the atrium and ventricle, including the spongy layer of the ventricle, when cardiac output, power output and oxygen consumption are doubled. As so many factors are increasing in exercise it is not possible from the present results firmly to ascribe the stimulation of protein synthesis to any particular factor. As mechanical stretching has been shown to stimulate protein synthesis in skeletal and cardiac muscle *in vitro* (Petersen & Lesch, 1972; Goldspink, 1981; Kira *et al.* 1984; Palmer *et al.* 1981), a working hypothesis would be that this is also the factor responsible for the stimulation of protein synthesis in trout hearts. Although the prostaglandins have been implicated in the response of skeletal muscle to stretch (Reeds & Palmer, 1986), recent work with rat hearts found no evidence for the involvement of these compounds in the pressure overload stimulation of protein synthesis (Smith & Sugden, 1987).

The fish heart is remarkable for the rapidity with which fractional rates of protein synthesis were stimulated by increased work load. In mammalian skeletal muscle, protein synthesis rates decrease during an exercise bout of less than 30 min and only clearly start to increase after 7 h of exercise (Booth & Watson, 1985).

Mammalian hearts show a stimulation of protein synthesis only during the second hour of pressure overload (Kira *et al.* 1984).

Support for the idea that the elevated atrial protein synthesis rates *in vitro* are an artefact of the *in vitro* preparation (see above) comes from two sources. First, *in vivo* there are no significant differences between the fractional protein synthesis rates in the atrium and the ventricle. Second, the RNA to protein ratios from both *in vitro* and *in vivo* hearts show that the bulbus arteriosus has the highest values (Figs 5, 6). D. N. McMillan & D. F. Houlihan (in preparation) have found a close correlation between fractional synthesis rates in different tissues of the trout and RNA to protein ratios; higher RNA to protein ratios are correlated with higher fractional rates of protein synthesis. From these observations we would expect the bulbus arteriosus to have the highest rate of synthesis of all the chambers. This was indeed found to be the case for the *in vivo* hearts but not for the *in vitro* bulbus arteriosus.

The increases in fractional rates of protein synthesis were brought about by increases in the activity of RNA in the atrium and ventricle. Since in various muscle types 85–94 % of cellular RNA is ribosomal (Young, 1970), relatively short-term increases in power output in the trout heart may be increasing the efficiency of ribosomal translation. In rat hearts, increases in aortic pressure bring about an increase in ribosomal protein synthesis after 1 h (Balvin *et al.* 1987) and an increase in RNA content is an early event in pressure overload hypertrophy in mammals (Morgan, Rannels & McKee, 1979).

The bulbus arteriosus was the only chamber of the heart which showed no significant change in fractional rate of protein synthesis with exercise either *in vitro* or *in vivo*. On ventricular contraction the bulbus expands to accommodate nearly the entire ventricular ejaculate (Johansen & Gesser, 1986) and during swimming the increased cardiac output may result in a significantly increased stretching of the bulbus accompanying the increase in ventral aortic pressure (Kiceniuk & Jones, 1977). Therefore, either the bulbus is not subject to the stimuli which elevate the rate of protein synthesis in the atrium and ventricle or the relatively high rate of synthesis in the bulbus is insensitive to these stimuli in the short term. The bulbus is composed mainly of smooth muscle and elastica (Santer, 1985) and Lewis *et al.* (1984) found in rats that the fractional rates of protein synthesis have the following trend: smooth muscle > heart and slow-twitch muscle > fast-twitch muscle. The high fractional rates of protein synthesis in the bulbus arteriosus may therefore be due to the inherently higher rates of protein synthesis in smooth muscle compared with cardiac muscle.

Coronary circulation and the compact layer of the ventricle

The role of the coronary circulation in salmonids has been the subject of some doubt (Daxboeck, 1982; Farrell & Steffensen, 1987). The results of the present experiments demonstrate that *in vitro* cardiac performance is improved when the coronary artery is perfused, although the effects were only significant in terms of cardiac output and input pressures. Also, *in vitro* the fractional rate of protein

synthesis was increased in the compact layer in low-work hearts. This points either to the compact layer not being adequately flooded by diffusion of phenylalanine from the perfusion fluid through the spongy layer of the ventricle in hearts without coronary perfusion or to the improved oxygen supply increasing the fractional rate of protein synthesis in the perfused compact layer. In low-work hearts with coronary perfusion the fractional rate of protein synthesis in the compact muscle was increased so that it was not significantly different from that of the spongy layer.

The *in vivo* experiments clearly show that the compact layer contributes much more to the total rate of protein synthesis in the ventricle than the *in vitro* experiments would lead one to expect (Table 2). These results point to the value of investigating physiological functions both *in vivo* and *in vitro*. In swimming fish the compact layer showed the greatest increase in fractional protein synthesis rate (1·5-fold increase compared with approximately 1·3-fold increases in the whole ventricle and the ventricular spongy layer). As the results from the present study point to the force of muscle contraction and the rate of protein synthesis being intimately linked, increased protein synthesis in the compact layer may be associated with increased muscle activity. Hence, with increased swimming speed the compact layer of the ventricle may become the principal site of protein synthesis in the ventricle.

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