the activity of the enzyme but only enhance the permeability of ATP through the membrane to the active enzyme centre.

The activity of Mg^{++} dependent ATPase in the erythrocyte membrane is enhanced in addition to insulin by adrenaline or noradrenaline which influence the contraction of smooth muscle. Does this fact indicate a certain relation of Mg^{++} dependent ATPase to contractile ghost protein[12]? A working hypothesis is considered that Mg^{++} dependent ATPase could form a part of the region of the pores, by which some substances penetrate through the membrane.

Zusammenfassung. Die Hormone Adrenalin oder Noradrenalin stimulieren in menschlichen Erythrozyten die Aktivität der Mg^{++}-abhängigen ATPase. Die Aktivität dieses Enzyms ist auch nach der Wirkung des Insulins gesteigert.

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Protein Synthesis in Isolated, Beating Rat Atria

To study further the pathogenesis of cardiac hypertrophy[1–4], and to increase understanding of the control of cardiac protein synthesis, it would be useful to be able to study the latter process in vitro. The spontaneously beating rat atrium lends itself to such studies, particularly to investigation of interventions affecting cardiac protein synthesis over short time periods.

Methods. Male, Sprague-Dawley rats were anesthetized with ether and their hearts quickly removed. Both atria were separated from the ventricles and placed into 25 ml Erlenmeyer flasks. This procedure required less than 2 min. 5 ml of Tyrode's medium containing glucose (5 µm/ml) and L-leucine (1.0 µm/ml) served as the incubation medium. L-leucine-1-^{14}C was added to yield a final specific activity of 0.25 µc/µm. The effect on incorporation rates of variation in leucine concentration was examined by also incubating atria with 0.25, 0.50, or 4.0 µm/ml leucine, with the specific radioactivity held constant. The medium was equilibrated before use with 95% O_2/5% CO_2 to yield a pH of 7.4 at 37°C, 7.2 at 30°C and 7.0 at 22°C. At the end of incubation the atria were homogenized and total atrial protein was prepared for radioassay as described elsewhere[5].

The rate of leucine uptake from the medium was determined as follows: after preincubation for 1 h in non-isotopic medium, the atria were transferred to medium containing leucine-1-^{14}C and allowed to incubate for either 10 or 30 min. The tissue was then rinsed quickly 3 times, blotted and homogenized in exactly 3.0 ml of 5% TCA. Protein was precipitated for at least 3 h at 0–4°C. A 0.5 ml aliquot of the supernate was radioassayed in a liquid scintillation system. Paper chromatography confirmed that all counts were in ^{14}C-leucine. The increment in activity in the 30 min over the 10 min sample confirmed that uptake of leucine had occurred. Further 15 min uptake experiments then were used for the data reported herein.

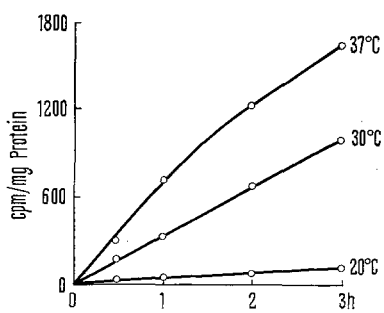

Fig. 1. The dependence of total atrial protein synthesis upon incubation time and temperature. Note that at each temperature protein synthesis is linear for at least 2 h. In the 37 °C system, there may be some fall in rate by the 3rd h of incubation.

Results. The linear incorporation of leucine into atrial protein as a function of time at various temperatures is shown in Figure 1. The relatively high rate of isotope incorporation into atrial protein at 30°C, compared with that in rat ventricular, kidney and liver slices and in whole rate diaphragm, is shown in Table I. The differences do not seem due solely to differences in leucine uptake (Table II). The effect on isotope incorporation of varying concentrations of leucine in the medium is shown in Figure 2. Above 0.5 µm/ml the final specific activity of total atrial protein is maximal. Progressive increments in leucine uptake occur at each comparable concentration (Figure 3). Protein synthesis is about 25%

Table I. Incorporation of leucine-1-^{14}C into total protein by various rat tissue preparations

Tissue	Specific activity (cpm/mg protein)
Ventricular slices	76 ± 10[a]
Intact diaphragm	116 ± 12
Kidney slices	278 ± 23
Liver slices	423 ± 18
Intact, beating atria	623 ± 50

[a] Mean ± S.E. of the mean.

Table II. Uptake of leucine-1-^{14}C from the incubation medium by various rat tissue preparations

Tissue	Uptake (µm × 10^3 leucine/100 mg wet wt.)
Kidney slices	57 ± 1.5[a]
Liver slices	45 ± 1.5
Intact, beating atria	51 ± 1.5

[a] Mean ± S.E. of the mean.

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more rapid in atria from animals weighing 75–100 g than in those weighing 200–250 g (Table III). The corresponding uptake data are shown.

Discussion. These rat atria beat spontaneously for several hours and incorporate ^{14}C-leucine into protein linearly during these time periods. Incorporation is 90% inhibited by $10^{-4}M$ puromycin indicating that true peptide bond formation has occurred. Incorporation is temperature-dependent but the differences in rate seen at 20°, 30° and 37°C are unlikely to be the result of the small pH differences at these various incubation temperatures. At concentrations of leucine in the medium above 0.5 µm/ml, the protein synthetizing apparatus appears to be saturated since the rate of protein synthesis is maximal in the face of increasing leucine uptake. To test the possibility that the rate of leucine incorporation is maximal because of some limiting 'toxic' effect of the isotope present, the specific radioactivity of the leucine in the medium was increased 10-fold. This manipulation resulted in a corresponding 10-fold increase in the final specific activity of total atrial protein isolated after incubation.

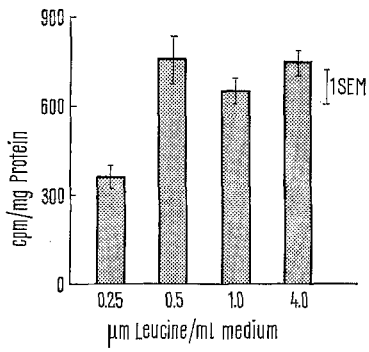

Fig. 2. The effect on atrial protein synthesis of various leucine concentrations in the incubation medium. The rate is reduced in concentrations below 0.5 µm leucine/ml. The variations above this concentration are not significantly different from one another.

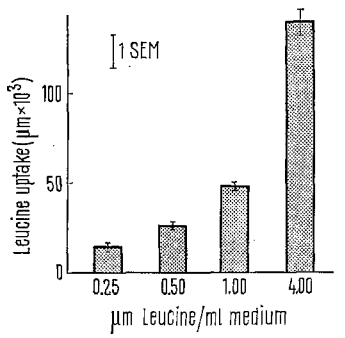

Fig. 3. The 15 min atrial uptake of leucine-1-^{14}C from incubation media containing different concentrations of cold leucine.

Table III. Incorporation into protein and tissue uptake of ^{14}C-leucine by atria from young vs. older animals

	Leucine incorporation (cpm/mg)	Leucine uptake (µm × 10^3/ 100 mg wet wt.)
Young	837 ± 64.1[a]	58.5 ± 2.5
Older	682 ± 14.8	45.0 ± 1.5

[a] Mean ± S.E. of the mean.

The rate of leucine incorporation into atrial protein is at least as rapid as that observed in liver and kidney slices and exceeds the rate for diaphragm. It is much more rapid than the rate for ventricular slices, suggesting that atria may be considerably more useful than such ventricular slices for a variety of cardiac metabolic studies, in addition to protein synthesis. Although the differing rates of leucine incorporation among these tissues do not seem to be the result of differences in leucine uptake, they could be due to differences in tissue leucine pool size. This possibility is still to be explored. The data also suggest that the more rapid incorporation of leucine by atria from young versus older animals cannot be explained on the basis of differences in uptake. At the leucine concentration used in these studies, the rate of atrial protein synthesis appears independent of small changes in leucine uptake.

The question of whether net new protein synthesis occurs during incubation of the atria is not yet answered. However, in preliminary experiments we have studied $^{14}CO_2$ production by atria prelabelled with ^{14}C-leucine in the presence and in the absence of puromycin. The rates of $^{14}CO_2$ production are low and similar in these two systems, indicating that there is relatively little $^{14}CO_2$ produced when the protein has become labelled. This finding suggests that little catabolism of protein occurs during such short-term studies and, although tentative and indirect, this evidence suggests that the incorporation seen represents synthesis rather than merely turnover of protein.

It is difficult to know how closely energy stores in these atria match the levels present in vivo. However, ATP and creatine phosphate contents of these atria measured after incubation are sufficient to maintain vigorous beating at rates approximating those in vivo (125–150 beats/min)[6]. Furthermore, such atria do not produce measurable lactate during incubation, again suggesting that relatively normal tissue energetics prevail.

Thus, isolated rat atrium seems a useful and convenient tissue for in vitro study of protein synthesis in heart muscle. Protein synthesis is linear, reasonably rapid, and appears to proceed under conditions of reasonably normal tissue energetics. The system seems particularly useful for studying processes which might affect protein synthesis in heart muscle over short time periods[7].

Zusammenfassung. Untersuchungen über die Proteinsynthese am isolierten, spontan schlagenden Rattenherzvorhof ergeben einen linearen Syntheseverlauf während mehrerer Stunden bei relativ normalem Energieverbrauch des Gewebes. Die Einbaugeschwindigkeit des Leuzins in das Gesamtprotein des Vorhofs ist höher als bei anderen untersuchten Geweben.

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