The effects of lactate, acetate, glucose, insulin, starvation and alloxan-diabetes on protein synthesis in perfused rat hearts

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Compared with glucose, lactate + acetate stimulated ventricular protein synthesis in anterogradely perfused hearts from fed or 72 h-starved rats. Stimulation was greater on a percentage basis in starved rats. Atrial protein synthesis was not detectably stimulated by lactate + acetate. Insulin stimulated protein synthesis in atria and ventricles. The stimulation of protein synthesis by lactate + acetate and insulin was not additive, the percentage stimulation by insulin being less in the ventricles of lactate + acetate-perfused hearts than in glucose-perfused hearts. Perfusion of hearts from 72 h-starved or alloxan-diabetic rats with glucose + lactate + acetate + insulin did not increase protein-synthesis rates or efficiencies (protein synthesis expressed relative to total RNA) to values for fed rats, implying there is a decrease in translational activity in these hearts. In the perfused heart, inhibition of protein synthesis by starvation and its reversal by re-feeding followed a relatively prolonged time course. Synthesis was still decreasing after 3 days of starvation and did not return to normal until after 2 days of re-feeding.

INTRODUCTION

In the Langendorff (1895)-perfused heart, ventricular protein-synthesis rates are increased by non-carbohydrate fuels (long-chain fatty acids, ketone bodies, acetate, lactate and pyruvate) or insulin compared with hearts perfused with glucose alone (Morgan *et al.*, 1971; Rannels *et al.*, 1974; Flaim *et al.*, 1983). Much of the stimulation could be attributed to the removal of a 'perfusion-induced' inhibition of protein synthesis at the level of initiation occurring after about 1 h (for a review, see Morgan *et al.*, 1979). The inhibition has been attributed to removal of bound insulin or exhaustion of endogenous triacylglycerol stores. However, in the anterogradely perfused heart (in which conditions *in vivo* are more closely simulated) with glucose as fuel, inhibition of protein synthesis does not develop. Rates are greater than in Langendorff-perfused hearts in the first 1 h of perfusion and are linear for 2 h (Morgan *et al.*, 1980; Smith & Sugden, 1983a).

Although ventricular protein turnover has been extensively investigated, atrial turnover has been relatively ignored. Atrial synthesis rates are approx. 2-fold greater than ventricular rates *in vitro* and *in vivo*, and are less affected by starvation, insulin or hypoxia (Smith & Sugden, 1983a; Preedy *et al.*, 1985a,b). It was therefore decided to investigate whether non-carbohydrate fuels stimulated protein synthesis in atria and ventricles of the anterogradely perfused heart and to study the combined effects of non-carbohydrate fuels, insulin, starvation and diabetes.

EXPERIMENTAL

Materials and animals

Sources have been given previously (Preedy *et al.*, 1984). Male Sprague-Dawley rats (initial body wt.

250–350 g) were housed and fed as described in Preedy *et al.* (1984). In starvation and re-feeding experiments, rats were housed in grid-bottom cages. Food was removed or provided at 11:00 h. Diabetes was induced by injection of alloxan (60 mg/kg) in 0.15 M-NaCl into the lateral tail vein of conscious restrained rats 2 days before the experiment. Water intake in diabetic rats ($n = 24$) was 213 ± 12 ml/2 days (control = 72 ± 2 ml/2 days, $n = 11$) and final blood glucose concentration was 41.7 ± 2.0 mM (control = 8.0 ± 0.3 mM, $n = 6$).

Measurement of protein-synthesis rates in the anterogradely perfused rat heart

Hearts were anterogradely perfused by the method of Taegtmeier *et al.* (1980) as described in detail previously (Sugden & Smith, 1982b). Hearts were initially pre-perfused as non-recirculating Langendorff (1895) preparations at 10 kPa pressure with Krebs & Henseleit (1932) bicarbonate-buffered saline supplemented as appropriate with fuels and insulin (50 munits/ml). Lactate, acetate and propionate were added as their sodium salts (pH 7.4) prepared as described previously (Sugden & Smith, 1982a). Perfusate NaCl concentration was decreased to maintain constant Na^+ concentrations in these perfusions. All perfusates were equilibrated with O_2/CO_2 (19:1). After rapid cannulation of the left atrium, the heart was switched to an anterogradely perfused preparation where perfusate (100 ml) was continuously recirculated. The filling pressure was 0.5 kPa and the aortic pressure 7 kPa. The basic perfusate was the same as in the Langendorff pre-perfusion, but contained additionally 0.4 mM-[U- ^{14}C]phenylalanine (sp. radioactivity about 0.1 Ci/mol) and the other amino acids necessary for protein synthesis at a concentration of 0.2 mM. Under these conditions, rates of atrial and ventricular protein synthesis are linear for at least 2 h, and a further increase in amino acid concentration does not increase the rate of protein

Abbreviations used: k_s , rate of protein synthesis expressed relative to tissue protein, in units of pmol of phenylalanine incorporated/2 h per mg of protein; k_{RNA} , efficiency of protein synthesis, i.e. rate of protein synthesis expressed relative to tissue RNA, in units of pmol of phenylalanine incorporated/2 h per μg of RNA.

Table 1. Effects of fuels and insulin on protein synthesis in ventricles and atria from fed, 3-day-starved and diabetic rats

Hearts were perfused and protein-synthesis rates were measured as described in the Experimental section. When present, the insulin concentration was 50 munits/ml. Abbreviation: N.D., not determined. In Expt. 1, statistical significance: (a) for effects of insulin versus perfusions with identical fuels in rats of the same nutritional state, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; (b) for effects of fuels in the presence or absence of insulin versus perfusions with glucose alone, $\dagger P < 0.05$, $\ddagger P < 0.01$, $\S P < 0.001$ [significance with glucose as sole fuel as in (a)]; (c) for effects of starvation versus feeding in perfusions with identical fuels + insulin, $\dagger P < 0.05$, $\ddagger P < 0.01$, $\S P < 0.001$. Expt. 2 was carried out at a different time from Expt. 1. For Experiment 2, statistical significance of results for diabetic rats versus results for fed rats in perfusions containing glucose + lactate + acetate + insulin: $\$P < 0.05$, $\S\S P < 0.01$, $\S\S\S P < 0.001$.

Expt.	Nutritional state	Additions to perfusate	n	Ventricles		Atria	
				k_s (pmol of phenylalanine incorporated/2 h per mg of protein)	k_{RNA} (pmol of phenylalanine incorporated/2 h per μ g of RNA)	k_s (pmol of phenylalanine incorporated/2 h per mg of protein)	k_{RNA} (pmol of phenylalanine incorporated/2 h per μ g of RNA)
Expt. 1	Fed	5 mM-glucose	25	1215 $\pm$ 28	188 $\pm$ 6	2567 $\pm$ 71	184 $\pm$ 5
		5 mM-glucose + insulin	9	1895 $\pm$ 103***	296 $\pm$ 23***	3110 $\pm$ 204**	249 $\pm$ 11***
		10 mM-lactate + 10 mM-acetate	11	1744 $\pm$ 48†††	272 $\pm$ 20†††	2669 $\pm$ 187	194 $\pm$ 11
		20 mM-lactate	4	1874 $\pm$ 85†††	280 $\pm$ 19†††	2964 $\pm$ 126†	206 $\pm$ 9†
		20 mM-acetate	4	1876 $\pm$ 46†††	302 $\pm$ 23†††	2791 $\pm$ 115	191 $\pm$ 12
		10 mM-lactate + 10 mM-acetate + insulin	15	1965 $\pm$ 59* †††	273 $\pm$ 10†††	3181 $\pm$ 154†††	250 $\pm$ 10** †††
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate	8	1807 $\pm$ 64†††	N.D.	N.D.	N.D.
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	10	2059 $\pm$ 64* †††	N.D.	N.D.	N.D.
		5 mM-glucose	10	665 $\pm$ 29†††	108 $\pm$ 6†††	1549 $\pm$ 78††	138 $\pm$ 5†††
		5 mM-glucose + insulin	8	1307 $\pm$ 30*** †††	N.D.	1669 $\pm$ 122†††	N.D.
Expt. 2	Starved	10 mM-lactate + 10 mM-acetate	16	1498 $\pm$ 25††† †††	211 $\pm$ 4††† ††	2109 $\pm$ 54††† ††	151 $\pm$ 8††
		10 mM-lactate + 10 mM-acetate + insulin	11	1617 $\pm$ 47* ††† †††	244 $\pm$ 11** †††	2270 $\pm$ 61††† †††	170 $\pm$ 12††
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate	4	1277 $\pm$ 45††† †††	182 $\pm$ 6††† ††	1653 $\pm$ 124†††	146 $\pm$ 11†
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	6	1579 $\pm$ 67* ††† †††	235 $\pm$ 11*††† †	2414 $\pm$ 159††† †	182 $\pm$ 16†† ††
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	8	1845 $\pm$ 27	195 $\pm$ 5	2741 $\pm$ 75	174 $\pm$ 6
		5 mM-glucose + insulin	4	1615 $\pm$ 80§	178 $\pm$ 8	2698 $\pm$ 163	165 $\pm$ 6
		10 mM-lactate + 10 mM-acetate	5	1514 $\pm$ 37§§§	163 $\pm$ 4§§	2483 $\pm$ 79	152 $\pm$ 5§
		10 mM-lactate + 10 mM-acetate + insulin	5	1480 $\pm$ 67§§§	166 $\pm$ 7§§	2609 $\pm$ 94	152 $\pm$ 5§
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate	5	1484 $\pm$ 67§§§	164 $\pm$ 2§§	2324 $\pm$ 81§§	146 $\pm$ 5§§
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	5	1554 $\pm$ 73§§	170 $\pm$ 7§	2444 $\pm$ 126§	152 $\pm$ 5§
Expt. 2	Diabetic	5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	8	1845 $\pm$ 27	195 $\pm$ 5	2741 $\pm$ 75	174 $\pm$ 6
		5 mM-glucose + insulin	4	1615 $\pm$ 80§	178 $\pm$ 8	2698 $\pm$ 163	165 $\pm$ 6
		10 mM-lactate + 10 mM-acetate	5	1514 $\pm$ 37§§§	163 $\pm$ 4§§	2483 $\pm$ 79	152 $\pm$ 5§
		10 mM-lactate + 10 mM-acetate + insulin	5	1480 $\pm$ 67§§§	166 $\pm$ 7§§	2609 $\pm$ 94	152 $\pm$ 5§
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate	5	1484 $\pm$ 67§§§	164 $\pm$ 2§§	2324 $\pm$ 81§§	146 $\pm$ 5§§
		5 mM-glucose + 10 mM-lactate + 10 mM-acetate + insulin	5	1554 $\pm$ 73§§	170 $\pm$ 7§	2444 $\pm$ 126§	152 $\pm$ 5§
		5 mM-glucose	10	665 $\pm$ 29†††	108 $\pm$ 6†††	1549 $\pm$ 78††	138 $\pm$ 5†††
		5 mM-glucose + insulin	8	1307 $\pm$ 30*** †††	N.D.	1669 $\pm$ 122†††	N.D.
		10 mM-lactate + 10 mM-acetate	16	1498 $\pm$ 25††† †††	211 $\pm$ 4††† ††	2109 $\pm$ 54††† ††	151 $\pm$ 8††
		10 mM-lactate + 10 mM-acetate + insulin	11	1617 $\pm$ 47* ††† †††	244 $\pm$ 11** †††	2270 $\pm$ 61††† †††	170 $\pm$ 12††

Table 2. Time course for the effects of starvation and re-feeding on protein synthesis in ventricles and atria

Hearts were perfused with 5 mM-glucose and protein synthesis was measured as described in the Experimental section. Statistical significance versus fed rats: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; $n = 4-13$ in each group.

Nutritional state	Ventricles		Atria	
	k_s (pmol of phenylalanine incorporated/ 2 h per mg of protein)	k_{RNA} (pmol of phenylalanine incorporated/ 2 h per μ g of RNA)	k_s (pmol of phenylalanine incorporated/ 2 h per mg of protein)	k_{RNA} (pmol of phenylalanine incorporated/ 2 h per μ g of RNA)
Fed	1349 $\pm$ 31	202 $\pm$ 8	3080 $\pm$ 89	178 $\pm$ 8
1 day starved	994 $\pm$ 60***	128 $\pm$ 6***	2685 $\pm$ 106*	147 $\pm$ 5*
2 days starved	981 $\pm$ 43***	120 $\pm$ 6***	2347 $\pm$ 31***	116 $\pm$ 6***
3 days starved	757 $\pm$ 32***	111 $\pm$ 5***	1972 $\pm$ 67***	126 $\pm$ 5***
3 days starved, 1 day re-fed	1101 $\pm$ 11***	164 $\pm$ 6*	2475 $\pm$ 89**	178 $\pm$ 9
3 days starved, 2 days re-fed	1247 $\pm$ 54	185 $\pm$ 6	3000 $\pm$ 95	192 $\pm$ 2
3 days starved, 3 days re-fed	1324 $\pm$ 86	195 $\pm$ 6	2625 $\pm$ 116*	187 $\pm$ 8

synthesis (Smith & Sugden, 1983a). Cardiac outputs over the course of the perfusion were measured as previously described (Sugden & Smith, 1982b) and were similar for all perfusions. Changes in protein-synthesis rates could not therefore be ascribed to changes in rates of ATP utilization. After 2 h of anterograde perfusion, [U- 14 C]phenylalanine incorporation into atrial or ventricular protein and the specific radioactivity of [U- 14 C]phenylalanine in perfusates were measured as described previously (Smith & Sugden, 1983a,b).

Other methods

Protein was measured by the method of Gornall *et al.* (1949) with rat heart mixed protein as standard (Smith & Sugden, 1983a). RNA was measured by the method of Munro & Fleck (1969). Results are presented as means $\pm$ S.E.M. with the numbers of observations (n). Significance of differences between groups was tested by a two-tailed unpaired Student's t test, with values of $P < 0.05$ taken as being statistically significant.

RESULTS

Effects of non-carbohydrate fuels and insulin on protein-synthesis rates in hearts from fed, 72 h-starved and alloxan-diabetic rats

Lactate and acetate were routinely used as non-carbohydrate fuel because, unlike certain other non-carbohydrate fuels such as fatty acids, they allow the heart to maintain its output (Taegtmeyer *et al.*, 1980) and also because they are stable and easy to handle (see the Discussion section for a more detailed justification). Results are shown in Table 1. Since, in acute experiments involving rats of identical nutritional states, interventions are likely to exert their effects only at the level of translation (Morgan *et al.*, 1979), results are considered to be significant only if apparent in measurements of both k_s and k_{RNA} . This allows minor variations in RNA/protein ratios to be controlled. For perfusions with glucose or with glucose + insulin (50 munits/ml), results are similar to those previously reported (Smith & Sugden,

1983b; Preedy *et al.*, 1985a). Increasing the glucose concentration in the absence of insulin from 5 mM to 25 mM did not alter these rates in fed rats (results not shown).

Perfusion of hearts from fed rats with 10 mM-lactate + 10 mM-acetate stimulated ventricular k_s and k_{RNA} by about 45%, compared with hearts perfused with glucose alone (Table 1). Stimulation was about 100–125% in ventricles from starved rats. These findings are different from those of Kochel *et al.* (1984), who found that starvation was a prerequisite for stimulation of synthesis by non-carbohydrate fuels. However, atrial k_s and k_{RNA} were not stimulated. (The k_s result for atria from starved rats in the presence of lactate + acetate is considered to be spurious, since there was no corresponding increase in efficiency.) Thus only in perfusions with glucose alone, with fed rats, were protein-synthesis rates in strict proportion to the RNA/protein ratio. *In vivo*, there was no strict proportionality between atrial and ventricular k_s and RNA/protein ratios in fed or starved rats (Preedy *et al.*, 1985a), suggesting an influence of non-carbohydrate fuels and insulin on protein synthesis *in vivo*. When hearts from fed rats were perfused with 20 mM-lactate (Table 1), with 20 mM-acetate (Table 1), with 5 mM-lactate + 5 mM-acetate or with 25 mM-lactate + 25 mM-acetate, protein-synthesis rates were similar to rates observed in perfusions with 10 mM-lactate + 10 mM-acetate (results not shown). Similarly, increasing both lactate and acetate concentration to 25 mM in perfusions of hearts from starved rats had no effect on protein-synthesis rates (results not shown). In the presence of lactate + acetate, insulin significantly stimulated both ventricular k_s and k_{RNA} only in hearts from starved rats. However, the stimulation by insulin was small (8–16%), because of the stimulation by lactate + acetate. The presence of 5 mM-glucose in these perfusions did not materially alter these conclusions. The stimulatory effects of lactate + acetate and insulin in the presence of 5 mM-glucose were not additive in hearts from fed or starved rats.

We examined whether propionate stimulated protein synthesis, as reported by Chua *et al.* (1979b). Although

propionate is metabolized by the heart (Davis & Quastel, 1964; Peuhkurinen, 1982), it cannot support contraction, presumably because the maximum flux through the pathway converting it into succinyl-CoA is too low. We were unable to confirm that propionate stimulated protein synthesis. For fed rats, ventricular k_s in perfusions with 5 mM-glucose + 10 mM-propionate was 1163 ± 57 pmol of phenylalanine incorporated/2 h per mg of protein ($n = 7$; cf. perfusions with 5 mM-glucose, Table 1). [It should be noted that the protein-synthesis rate in the presence of propionate quoted in Table IV of Chua *et al.* (1979b) does not appear to be statistically different from controls, at least by an unpaired t test, in spite of claims to the contrary].

Because acetate increases acetyl-CoA and acetylcarnitine concentrations in hearts perfused with glucose plus insulin (McAllister *et al.*, 1973), we perfused hearts with 10 mM-glucose plus 10 mM-dichloroacetate (which causes increases in acetyl-CoA and acetylcarnitine concentrations similar to perfusion with glucose plus acetate, because of activation of pyruvate dehydrogenase; see McAllister *et al.*, 1973; Whitehouse *et al.*, 1974). However, there was no significant stimulation of protein synthesis in these perfusions (results not shown). Thus stimulation is unlikely to be mediated by acetyl-CoA or acetylcarnitine.

Lactate + acetate and/or insulin could not increase k_s and k_{RNA} in hearts from starved or diabetic rats to fed values (Table 1), even though in ventricles from starved rats the proportional stimulation by lactate + acetate compared with 5 mM-glucose perfusions was greater than in fed rats. (In our hands, hearts from diabetic rats cease pumping after a few minutes when glucose is the only fuel present.) There is a suggestion that diabetes inhibits atrial protein synthesis, but this is less than in ventricles. Thus the inhibition of heart protein synthesis observed in starved and diabetic rats *in vivo* (Pain & Garlick, 1974; Preedy *et al.*, 1984) is still apparent *in vitro* and cannot be overcome by non-carbohydrate fuels and insulin.

Time course of changes in protein-synthesis rates on starvation and re-feeding

k_s and k_{RNA} were decreased in atria by 13% and in ventricles by 26% after 1 day of starvation (Table 2). Thereafter k_s values decreased in both compartments and were presumably still decreasing after 3 days of starvation. After 1 day of food being available, synthesis rates in both atria and ventricles were about 80% of rates for fed animals. After 2 days of food being available, rates were not significantly different from those for fed rats. The time course of these changes in protein synthesis rates seems slower than in gastrocnemius muscle *in vivo* (Garlick *et al.*, 1983). Starvation for 12 h decreased gastrocnemius protein synthesis by 41% (cf. 3-day-starved rats, Table 2). Protein synthesis was 85% of the value for the fed control after 1 h of re-feeding (cf. 1-day-re-fed rats, Table 2). No results of re-feeding experiments *in vivo* for the heart have yet been reported, to our knowledge.

DISCUSSION

Concentrations of non-carbohydrate fuels and insulin in perfusates in relation to concentrations *in vivo*

We used acetate in perfusions because we consider it to be a long-chain fatty acid analogue without being as

difficult to handle as long-chain fatty acids themselves. Taegtmeier *et al.* (1980) suggested that long-chain fatty acids would not allow cardiac output to be maintained in prolonged perfusions in the working heart. A further difficulty is that the albumin present in the perfusions causes considerable frothing. The plasma long-chain fatty acid concentration in starved animals is about 1 mM. In terms of acetyl-group provision, this is equivalent to an acetate concentration of about 8 mM. Lactate concentrations used were higher than those in the plasma (about 1–2 mM). However, it is likely that plasma [lactate] is lower than tissue [lactate]. Chua *et al.* (1979a) showed that, in hearts perfused the absence of lactate, the intracellular lactate concentration was 3 mM. Furthermore, in hearts perfused with lactate, intracellular lactate concentrations were only about 60% of extracellular values. It is thus to be expected that perfusion of hearts with 10 mM-lactate would produce rises in intracellular [lactate] close to those encountered physiologically. Concentrations of insulin were grossly hyperphysiological in order to ensure a maximal response. However, we have shown maximum stimulation of cardiac protein synthesis *in vitro* at physiological insulin concentrations (S. J. Fuller & P. H. Sugden, unpublished work).

Effects of non-carbohydrate fuels and insulin on protein synthesis in the heart

Perfusion of working hearts from fed or 72 h-starved rats with non-carbohydrate fuels stimulated ventricular protein synthesis compared with hearts perfused with glucose alone (Table 1). Stimulation of protein synthesis was not the result of prevention of development of the perfusion-induced inhibition of protein synthesis, for, in working hearts, protein-synthesis rates are linear for at least 2 h and initial rates of protein synthesis are greater than in Langendorff-perfused hearts (Morgan *et al.*, 1980; Smith & Sugden, 1983a). Non-carbohydrate fuels thus have an 'insulin-like' effect on ventricular protein synthesis, for their stimulatory action is nearly as great or is as great as that of insulin (Table 1). However, these experiments do not necessarily indicate that insulin and non-carbohydrate fuels stimulate protein synthesis by a common mechanism, especially since atrial protein synthesis is stimulated by insulin, but not by non-carbohydrate fuels. The reason for this lack of stimulation in atria is unclear. In the hemicorpus, perfusion with palmitate did not stimulate psoas or gastrocnemius muscle protein synthesis compared with glucose-perfused controls (Jefferson *et al.*, 1972; Li & Jefferson, 1978). This could result from the relative inability of these fast-twitch muscles to oxidize fatty fuels. However, this is unlikely to be the case for atrial muscle, since these fibres contain slightly more mitochondria than do ventricular fibres (Fawcett & McNutt, 1969; McNutt & Fawcett, 1969), so should be capable of oxidizing non-carbohydrate fuels.

Effects of starvation and diabetes on protein-synthesis rates

Perfusion of hearts from 72 h-starved rats with lactate + acetate + insulin did not increase k_s and k_{RNA} to values for fed rats (Table 1). *In vivo*, starvation causes a decrease in cardiac protein mass (Preedy *et al.*, 1984). Protein synthesis is inhibited, but inhibition is not as great on a percentage basis as in gastrocnemius (Garlick *et al.*, 1975). Rates of protein synthesis in hearts of starved rats

in vivo are greater than in glucose-perfused hearts *in vitro* (Preedy *et al.*, 1984) and are similar to rates in hearts perfused with lactate+acetate *in vitro* [Table 1; heart protein phenylalanine content by amino acid analysis being 296 ± 4 nmol/mg, $n = 7$ (P. H. Sugden, unpublished work)]. This suggests that, although cardiac protein is lost and protein synthesis is inhibited by starvation, loss of protein and inhibition are not as great as they would be in the absence of non-carbohydrate fuels. A problem with this argument is that atrial protein synthesis is relatively unaffected by non-carbohydrate fuels, which is not to be expected if these fuels are important in the maintenance of cardiac mass. However, atrial protein synthesis is less severely affected by starvation than is ventricular protein synthesis (Table 1; Preedy *et al.*, 1985a). Unequivocal evidence as to the physiological importance of non-carbohydrate fuels in maintaining protein synthesis rates requires appropriate experiments to be carried out *in vivo*.

The effect of diabetes on cardiac protein synthesis is controversial. Rannels *et al.* (1970, 1974) have suggested that k_{RNA} is protected from the effects of 2 days of alloxan-diabetes and any inhibition results from loss of RNA. In contrast, Pain & Garlick (1974) have shown a decrease in cardiac k_{RNA} by 5 days of streptozotocin-diabetes *in vivo*. Subsequently, Williams *et al.* (1980) showed a decrease in RNA/protein ratio and k_{RNA} in alloxan-diabetic insulin-maintained and -withdrawn rats. Our results show that, although inhibition of protein synthesis in diabetes or starvation was partly attributable to a decrease in RNA/protein ratios (Preedy *et al.*, 1984, 1985a) [alloxan-diabetes slightly decreased ventricular RNA/protein ratios from 9.58 ± 0.10 $\mu\text{g}/\text{mg}$ ($n = 6$) to 9.09 ± 0.07 $\mu\text{g}/\text{mg}$ ($n = 27$, $P < 0.001$), but did not alter atrial ratios], both atria and ventricles showed significant decreases in k_{RNA} .

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