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Effect of Adenosine on the Adenine Nucleotide Content and Metabolism of Hepatocytes

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1. Adenosine (0.5mM) added to hepatocyte suspensions increased the intracellular concentration of ATP and total adenine nucleotides within 60min up to three-fold. 2. Adenosine at 0.5mM inhibited gluconeogenesis from lactate by about 50%. At higher adenosine concentrations the inhibition was less. There was no strict parallelism between the time-course of the increase of the adenine nucleotide content and the time-course of the inhibition of gluconeogenesis from lactate. 3. Adenosine abolished the accelerating effects of oleate and of dibutyryl cyclic AMP on gluconeogenesis from lactate. 4. Gluconeogenesis from pyruvate and glutamine was also inhibited by the addition of adenosine but there was no significant effect of adenosine with fructose, dihydroxyacetone or glycerol. With asparagine, adenosine caused an acceleration of glucose formation. 5. Adenosine incorporation into adenine nucleotides accounted for about 20% of the adenosine removal. Most of the adenosine was deaminated as indicated by the formation of NH_3 and urea. 6. Inosine, hypoxanthine or adenine compared with adenosine gave relatively slight increases of adenine nucleotides. 7. Urea synthesis from NH_4Cl under optimum conditions i.e. in the presence of ornithine, lactate and oleate, was also inhibited by adenosine. The inhibition increased with the adenosine concentration and was 65% at 4mM-adenosine. Again there was no correlation between the degree of inhibition of urea synthesis and the increase in the adenine nucleotide content. 8. The basal O_2 consumption, the increased O_2 consumption on the addition of oleate and the rate of formation of ketone bodies were not affected by the addition of adenosine. The [β-hydroxybutyrate]/[acetoacetate] ratio was increased by adenosine, provided that lactate was present. 9. The increase of the adenine nucleotide content of the hepatocytes on the addition of adenosine may be explained on the assumption that adenosine kinase is not regulated by feedback but by substrate supply.

In the course of experiments on the effect of nitrogenous substances on gluconeogenesis from lactate in isolated hepatocytes (see Cornell *et al.*, 1974) it was noted that adenosine inhibits gluconeogenesis and at the same time it increases the intracellular concentration of ATP and total adenine nucleotides about threefold. As the ATP and total adenine nucleotide content of tissues are normally maintained within close limits *in vivo*, the concentrations reached in the presence of adenosine are surprisingly high. The present paper is a follow up of these effects of adenosine.

Materials and Methods

Rats

Female Wistar rats (180–220g) were obtained from Charles River U.K. Ltd., Manston Road, Margate, Kent, U.K. and were fed on Oxoid (Oxoid Ltd., London S.E.1, U.K.) pasteurized breeding diet for rats and mice. For some experiments, animals were starved for 48h before use.

Preparation of isolated liver cells

Cells were prepared essentially as described by Berry & Friend (1969) with the modifications described by Cornell *et al.* (1973) and Krebs *et al.* (1974). The number of observations given in the Tables for any given experimental condition refer to separate batches of cells.

Preparation of isolated kidney tubules

Kidney tubules were prepared as described by Guder & Wieland (1971).

Reagents

Enzymes and coenzymes were products of Boehringer Corp. (London) Ltd., London W.5, U.K. Other chemicals were reagent grade.

Determination of metabolites

Glucose was determined by the method of Slein (1963), lactate and pyruvate by those of Hohorst *et al.* (1959), 3-hydroxybutyrate and acetoacetate by

those of Williamson *et al.* (1962), ATP by that of Lamprecht & Trautschold (1963), and ADP and AMP by those of Adam (1963). The ATP assay was modified by using 2.5mM-glucose instead of the recommended 40mM because glucose–NADP reductase present in some glucose 6-phosphate dehydrogenase preparations causes a creep at the high glucose concentrations. NH_3 and urea were determined successively in the same cuvette in the assay system described by Bernt & Bergmeyer (1974) for urea. Free adenosine remaining after the incubations was measured by the change in extinction at 265nm after treatment of the sample with adenosine deaminase (Moellering & Bergmeyer, 1970). O_2 consumption in the presence of 5% CO_2 was measured manometrically (for details see Krebs *et al.*, 1974).

Results

Inhibition by adenosine of gluconeogenesis from lactate

The inhibition of gluconeogenesis from lactate and the accumulation of ATP increased with the adenosine concentration up to 0.5mM-adenosine (Table 1). At this concentration the inhibition was 50%. At higher adenosine concentrations the inhibition decreased to about 30% at 2mM. The inhibition was even greater in the presence of oleate (1mM) which in the absence of adenosine, like other fatty acids, accelerates gluconeogenesis from lactate (Krebs *et al.*, 1963, 1965; Struck *et al.*, 1965) (probably by supplying acetyl-CoA for maximum activation of pyruvate carboxylase). This accelerating effect of oleate was virtually abolished by adenosine; as a result the inhibition by adenosine approached 70% in the presence of oleate (Table 1).

Dibutyryl cyclic AMP also is known to be an accelerator of gluconeogenesis from lactate (Exton & Park, 1968). This effect was abolished by adenosine (Table 2). Likewise the additive accelerating effects of oleate and dibutyryl cyclic AMP disappeared in the presence of adenosine (Table 2). Thus in the presence of the accelerators of gluconeogenesis, adenosine depressed the rate of gluconeogenesis to that observed with lactate and adenosine.

It is noteworthy that added dibutyryl cyclic AMP (0.1mM) increased the intracellular concentration of ATP (Table 2), the cyclic AMP acting as the precursor. Oleate prevented this increase and also decreased the effectiveness of adenosine in raising

Table 1. *Effect of adenosine concentration on ATP content and on rates of gluconeogenesis from lactate in isolated liver cells*

Cells were prepared from livers of rats starved for 48h. Concentrations were initially 10mM-lactate and 1mM-oleate. The cell concentration was about 70 mg wet wt. in 4 ml. Incubation was for 60 min at 37°C. Values are means ± S.E.M. of four experiments.

	Lactate		Mean inhibition by adenosine	Lactate+oleate		Mean inhibition by adenosine
Adenosine (mM)	Glucose formed (µmol/min per g)	ATP (µmol/g)	(%)	Glucose formed (µmol/min per g)	ATP (µmol/g)	(%)
	0.65±0.04	2.02±0.092		1.25±0.133	2.06±0.096	
0.05	0.58±0.05	2.92±0.098	11	1.03±0.134	2.59±0.145	18
0.10	0.52±0.06	3.41±0.120	20	0.81±0.11	3.14±0.142	35
0.50	0.34±0.03	5.68±0.356	48	0.39±0.04	4.55±0.287	69
0.75	0.38±0.03	5.48±0.374	42	0.41±0.06	4.43±0.397	67
1.0	0.41±0.03	5.58±0.330	37	0.46±0.06	4.44±0.398	63
2.0	0.46±0.04	5.08±0.261	29	0.54±0.09	4.56±0.507	57

Table 2. *Inhibition by adenosine of the stimulatory effects of oleate and cyclic AMP on gluconeogenesis from lactate*

The initial concentrations were 10 mM-lactate, 1mM-oleate and 0.1mM-dibutyryl cyclic AMP. Each flask contained 80mg wet wt. of hepatocytes in 4ml. Incubation was for 60min at 37°C. The results are those of a representative experiment.

	Glucose formed (µmol/min per g)		ATP content (µmol/g)	
Additions	No adenosine	With 0.5mM-adenosine	No adenosine	With 0.5mM-adenosine
Lactate	0.61	0.31	1.93	5.77
Lactate, oleate	1.18	0.38	2.04	4.46
Lactate, cyclic AMP	0.87	0.34	3.11	6.24
Lactate, oleate, cyclic AMP	1.44	0.38	2.15	4.59

Table 3. *Time-course of increases in adenine nucleotides*

Liver cells from 48h-starved rats were used. The initial concentrations were 10mM-lactate and 0.5mM-adenosine. Extra phosphate was added as NaH_2PO_4–Na_2HPO_4, pH7.4. Analyses were carried out on extracts of the whole suspension. Separate experiments (see the text) showed the adenine nucleotides were totally intracellular. Values are means±S.E.M. of three experiments.

Additions	Incubation time (min)	ATP (μmol per g wet wt.)	ADP (μmol per g wet wt.)	AMP (μmol per g wet wt.)	Mean increases of total adenine nucleotides by adenosine (%)
Lactate	0	1.85±0.10	0.65±0.18	0.24±0.04	
	20	2.07±0.07	0.65±0.12	0.26±0.03	
	40	1.74±0.03	0 65±0.17	0.25±0.03	
	60	2.03±0.03	0.61±0.21	0.23±0.03	
Lactate+adenosine	20	3.63±0.10	1.12±0.23	0.49±0.06	76
	40	4.36±0.53	1.04±0.19	0.44±0.03	120
	60	6.01±1.10	1.21±0.11	0.41±0.00	162
Lactate+extra phosphate (4mM)+ adenosine	20	3.53±0.45	1.03±0.18	0.45±0.01	69
	40	5.66±0.32	1.29±0.21	0.45±0.03	179
	60	7.17±0.54	1.07±0.10	0.41±0.01	201
Lactate+extra phosphate (9mM)+ adenosine	20	3.96±0.08	1.00±0.18	0.45±0.04	82
	40	5.49±0.40	1.18±0.21	0.50±0.02	171
	60	7.69±0.52	1.16±0.06	0.42±0.05	222

the ATP concentration under other conditions. Changes in the concentration of intracellular adenine nucleotides should be borne in mind when cyclic AMP and related compounds are added to tissue preparations.

Time-course of the increases in ATP, ADP and AMP on incubation with adenosine

When hepatocytes were incubated with lactate the content of the three adenine nucleotides remained virtually constant over a period of 60min (Table 3). At 0.5mM-adenosine there was an increase of about 75% of the total adenine nucleotide content during the first 20min. At this early stage the increases of ADP and AMP closely paralleled those of ATP. During the later stages the increase of ATP became relatively greater. The rate of increase of total adenine nucleotide gradually decreased, and at 60min the increase was approximately threefold. The addition of extra phosphate to the incubation medium (4 or 9mM to give total concentrations of 5.2 and 10.2mM respectively) somewhat increased the rate between 20 and 60min. The increase after 60min was about fourfold with 9mM of extra phosphate. The relatively large S.E.M. variation of the ADP value in these experiments was due to the presence of a relatively large amount of pyruvate formed from lactate, which interferes with the precision of the determination of ADP.

The omission of lactate did not affect the increases in the adeninine nucleotide content caused by adenosine, but oleate decreased the effectiveness of adenosine when present as sole substrate.

Distribution of adenine nucleotides between cells and incubation medium

For the calculation of tissue concentrations it was necessary to know whether the newly synthesized adenine nucleotides are released into the incubation medium. To test this, conditions giving maximum increases were chosen (0.5mM-adenosine with 9mM of extra phosphate). After incubation, cells were rapidly separated from the medium by centrifugation by the method of Hems *et al.* (1975). No adenine nucleotide was detectable in the incubation medium when the total intracellular content was 9μmol per g wet wt.

Time-course of the inhibition of gluconeogenesis from lactate on the addition of adenosine

Table 1 may suggest that there is a strict parallelism between the increase in adenine nucleotides and the inhibition of gluconeogenesis from lactate. However, further experiments on the time-course of gluconeogenesis show that the inhibition on the addition of adenosine was greatest during the 40–60min period during which the increment of adenine nucleotides was smallest. Extra phosphate, particularly at the highest concentration tested (9mM), increased the degree of inhibition of gluconeogenesis.

Effect of adenosine on glucose synthesis from various precursors

In 14 separate experiments on cells from starved rats the mean inhibition of gluconeogenesis from lactate by 0.5mM-adenosine was 36%. With pyruvate

Table 4. *Adenine nucleotide synthesis from various precursors in liver cells from 48h-starved rats*

All flasks contained 10mM-lactate initially. The initial concentration of adenosine was 0.5mM and of other purine precursors 0.5 and 2mM. The values obtained at the two concentrations were combined as they were of the same order. Glutamine, asparagine and NH_4Cl were present at 5mM and glycine at 1mM. Incubation was for 60min. Means ± S.E.M. are given for the number of experiments in parentheses. In calculating the percentage effects, the value for a given experimental condition was compared with the control in the same experiment. A minus sign indicates a decrease.

Precursor added	ATP (μmol per g wt.)	Increase of ATP (%)	Total adenine nucleotide (μmol per g wet wt.)	Increase of total adenine nucleotides (%)	Effect on gluconeogenesis (%)
None (14)	2.10±0.06		3.16±0.13		
Adenosine (7)	6.45±0.46	193±22	7.62±0.56	157±23	−38±7
Adenosine+9mM-phosphate (3)	7.69±0.52	279±24	9.27±0.56	222±37	−50±2
Adenine (7)	2.67±0.10	23±3	4.01±0.23	22±2	−23±4
Inosine (4)	2.43±0.16	16±8	3.60±0.21	16±8	+6±2
Inosine+asparagine (7)	2.57±0.15	34±9	4.24±0.29	37±10	
Inosine+NH_4Cl (6)	2.30±0.16	16±6	3.50±0.09	16±1	
Hypoxanthine (6)	2.09±0.16	6±2	3.62±0.15	12±5	−26±6
Hypoxanthine+asparagine (10)	2.37±0.11	17±2	3.94±0.17	18±3	
Hypoxanthine+NH_4Cl (8)	1.89±0.14	−8±3	3.74±0.17	11±44	
Glutamine+NH_4Cl+glycine (4)	2.40±0.19	11±5	3.42±0.20	14±4	

as the substrate, the inhibition by adenosine was somewhat higher (46%) and with glutamine it was somewhat lower (29%). There was no significant effect of adenosine with fructose, dihydroxyacetone or glycerol. In the presence of asparagine, adenosine caused an acceleration (26%) of glucose formation.

Rate of adenosine removal

The rate of adenosine removal, assuming linearity, was 0.47μmol/min per g when the initial adenosine concentration was 0.5mM. The rate of disappearance did not increase with increasing adenosine concentrations, but at 4mM-adenosine it was slightly lower (0.40μmol/min per g). Adenosine incorporation into the adenine nucleotides accounted for only about 20% of the adenosine removal. Measurements of the formation of NH_3 and urea indicated that the remainder was deaminated to inosine. A small proportion of the ribose moiety of inosine was converted into glucose. The maximum rate of glucose formation from added adenosine was 0.1μmol/min per g.

It follows from the above rates of adenosine removal that at cell concentrations much above 80mg in 4ml, the adenosine added at 0.5mM is liable to disappear completely in the course of an incubation period of 1h. In accordance with this was the observation that there was little or no inhibition of gluconeogenesis from lactate at 60min when 0.5mM-adenosine was added to a 4ml suspension containing 160mg of cells. The inhibition was re-introduced when the adenosine concentration was increased to 1mM.

Synthesis of adenine nucleotides from other precursors

Inosine, hypoxanthine and adenine were tested at 0.5 and 2mM. Since inosine and hypoxanthine require aspartate for the amination of the purine ring, asparagine or NH_4Cl, both potential precursors of intracellular aspartate in the liver, were added together with inosine or hypoxanthine. None of the three substances tested (Table 4) approached the effectiveness of adenosine as precursors of adenine nucleotides. Inosine plus asparagine caused a 37% and adenine a 22% increase; hypoxanthine plus asparagine gave a mean increase of 18%. The other substrate combinations shown in Table 4 gave very slight increases of uncertain significance. This includes the addition of the precursors for the synthesis of the purine-ring system *de novo* (glutamine, glycine, NH_4Cl). The addition of ribose (10mM) or glucose (40mM) did not increase adenine nucleotide synthesis from purine bases.

Hypoxanthine and adenine inhibited glucose synthesis from lactate, but somewhat less than did adenosine.

The experiments recorded in Table 4 were carried out on cells from starved rats. They were repeated with cells from fed rats because cells containing glycogen form phosphoribosyl pyrophosphate much more rapidly than glycogen-depleted cells and phosphoribosyl pyrophosphate might be expected to limit the rate of the synthesis of adenine nucleotide *de novo* and the synthesis from added purine bases. The final concentrations of ATP and total adenine nucleotides reached on incubation were about the same in the fed and starved states. But as cells from fed rats contain on average a little more ATP

(2.61 μmol/g) than cells from starved rats (2.10 μmol/g) the percentage increase in ATP and total adenine nucleotides was slightly lower in the fed state. The small changes observed with other precursors were similar in the fed and starved states.

Effect of adenosine on the O_2 consumption, ketone-body formation and the redox states of cytosolic and mitochondrial NAD^+ couples

The O_2 consumption was not affected by adenosine except when gluconeogenic precursors were present (Table 5). In this situation the O_2 consumption was decreased on account of the lower ATP requirements at lower rates of gluconeogenesis. The rates of ketone-body production from endogenous sources and from added oleate were also unaffected by adenosine whereas the value of the [β-hydroxybutyrate]/[acetoacetate] ratio was raised about two-fold provided that lactate was present. The [lactate]/[pyruvate] ratio was likewise raised by the addition of adenosine. This occurred with both 10 and 2mM added lactate.

Inhibition of urea synthesis by adenosine

Under conditions where the rate of urea synthesis is maximum, i.e. in the presence of NH_4Cl, ornithine, lactate and oleate, adenosine inhibited the synthesis of urea (Table 6) from about 23% at 0.5mM-adenosine to 65% at 4mM-adenosine. Unlike the inhibition of glucose synthesis from lactate (Table 1) the inhibition of urea synthesis increased with adenosine concentration. There was no correlation between the percentage inhibition of urea synthesis by adenosine and the increase in adenine nucleotide content.

Under the same experimental conditions adenine, inosine and hypoxanthine at 2mM did not affect the synthesis of urea.

Synthesis of adenine nucleotides in kidney tubules

When adenine nucleotide precursors (adenosine, adenine, inosine, hypoxanthine) were incubated with isolated kidney tubules the increases of adenine nucleotides were very small (Table 7). As in the liver, adenosine was most effective but the increase was only of the order of 50%. Adenosine, adenine and inosine prevented a decrease on incubation rather than increasing the adenine nucleotide content above the initial value.

Discussion

Rate of adenine nucleotide synthesis from various precursors

It is already known (see Wyngaarden & Kelley, 1972) that the liver possesses the enzymes for the synthesis *de novo* of purines as well as for the 'salvage reactions' by which purine bases and nucleotides formed by the degradation of nucleotides or nucleic

Table 5. *Effect of adenosine on O_2 consumption, ketone-body formation and the redox states of NAD^+ couples*

Each flask contained 81 mg wet wt. of liver cells. Incubation was for 60min at 37°C. Initial concentrations were: lactate, 10mM; pyruvate, 1mM; oleate, 1mM; lysine, 2mM, except where stated otherwise. The concentration of adenosine was 0.75mM. Lysine and pyruvate were added to abolish the lag period (see Cornell *et al.*, 1974). The results show a representative experiment.

Substrate added	[Lactate]/[pyruvate]		[3-Hydroxybutyrate]/[acetoacetate]		Total ketone bodies (μmol per flask)		O_2 uptake (μmol/min per g)	
	No adenosine	With adenosine	No adenosine	With adenosine	No adenosine	With adenosine	No adenosine	With adenosine
None			0.15	0.15	3.84	2.66	2.90	3.12
Oleate			0.65	0.70	8.09	7.24	4.01	4.08
Lactate, pyruvate, lysine	9.6	13.9	0.26	0.56	1.87	2.17	5.19	4.40
Lactate, pyruvate, oleate, lysine	18.8	24.1	0.61	1.51	5.12	5.68	6.35	5.15
Lactate (2mM), lysine	7.4	11.0	0.31	0.40	3.35	2.59		
Lactate (2mM), lysine, oleate	10.0	19.6	0.74	1.38	7.43	6.75		

Table 6. *Inhibition of urea synthesis by adenosine*

All flasks contained 40mg wet wt. of liver cells from 48h-starved rats and initially 10mM-lactate, 1mM-oleate, 10mM-NH_4Cl and 2mM-ornithine. Incubation was for 60min at 37°C. The data are from a representative experiment.

Adenosine added (mM)	Rate of urea synthesis (μmol/min per g)	Inhibition of urea synthesis by adenosine (%)	Rate of glucose synthesis (μmol/min per g)	Inhibition of glucose synthesis by adenosine (%)	ATP at end of incubation (μmol per g)	Total adenine nucleotide at end of incubation (μmol per g)
0	4.97		1.57		1.84	2.78
0.5	3.82	23	0.40	75	3.60	5.16
1.0	3.27	34	0.66	58	3.55	5.60
2.0	2.74	45	0.70	55	3.55	5.66
4.0	1.73	65	0.41	74	3.90	5.68

Table 7. *Effects of precursors on adenine nucleotide synthesis in isolated kidney tubules*

Kidney tubules were prepared from 48h-starved rats. Each flask contained 25mg wet wt. in 2ml. Incubation was for 45min at 37°C. The initial concentrations of adenosine and adenine were 0.5mM and of hypoxanthine and inosine 2mM. The concentration of extra phosphate was 5mM. Average values for two separate experiments are given. The experiments were carried out by Dr. Patrick Vinay.

Experimental condition	Precursor added	ATP (μmol per g)	ADP (μmol per g)	AMP (μmol per g)
Freshly isolated tubules	None	1.57	0.71	0.26
Incubated tubules	None	1.21	0.49	0.26
Incubated tubules	Phosphate	1.24	0.53	0.26
Incubated tubules	Adenosine, phosphate	1.88	0.76	0.25
Incubated tubules	Adenine, phosphate	1.62	0.59	0.23
Incubated tubules	Inosine, phosphate	1.78	0.88	0.20
Incubated tubules	Hypoxanthine, phosphate	1.44	0.66	0.28

acids can be reconverted into nucleotides. However, there was no information on the quantitative aspects of the various salvage reactions in the intact cell. The present experiments show that adenosine is by far the most ready precursor. An adenosine kinase which catalyses the reaction adenosine+ATP→AMP+ADP in animal tissues was first described by Caputto (1951).

Chagoya de Sanchez *et al.* (1972) found an increase in hepatic adenine nucleotides after intra-peritoneal injection of rats (200mg of adenosine/kg body wt.) but compared with the effects in isolated hepatocytes the increases were small; 17% after 60min, 30% after 120min. The presence of adenosine kinase has also been reported for cardiac muscle (Jacob & Berne, 1960; Tsuboi & Buckley, 1965; Liu & Feinberg, 1971; Maguire *et al.*, 1972). However, these reports do not provide quantitative data on the rates of adenine nucleotide synthesis comparable with the measurements reported in the present paper.

Control of adenine nucleotide concentration of tissues

The total concentration of adenine nucleotides in animal tissues is fairly constant *in vivo* although the relative concentrations of ATP, ADP and AMP may vary according to the physiological state of the tissue. It therefore seems surprising that on the addition of adenosine the concentrations of total hepatic adenine nucleotides can rise more than threefold. This implies that in the intact liver the concentration of adenosine is kept sufficiently low (presumably by the action of adenosine deaminase) to forestall the kind of effects observed in the present experiments. When adenine nucleotides are resynthesized by salvage reactions from intracellular breakdown products the concentrations of total adenine nucleotides would not change unless the degradation of RNA supplies major amounts of degradation products. The intestinal digestion of dietary nucleic acids and nucleotides probably goes beyond the stage of adenosine. The main process controlling the concentration of total adenine nucleotides is probably the feedback-regulated synthesis *de novo* from phosphoribosyl pyrophosphate, glutamine, glycine, 5,10-methenyltetrahydrofolate, bicarbonate and aspartate. The glycine and the methenyl group arise from carbohydrate via serine (see Henderson & Paterson, 1973).

Mechanism of adenosine effects

No satisfactory explanation can as yet be offered for the inhibition of energy-requiring processes in the liver when extra ATP is available. Considering that adenine and hypoxanthine also inhibit gluconeogenesis without a major increase in the adenine nucleotide content, it is possible that a breakdown product of adenosine causes the inhibition. It is noteworthy that the [ATP]/[ADP] ratio increases from the normal value of between 2 and 3 to about 7 on exposure to adenosine (whereas the basal O_2 uptake and rate of total ketone-body formation from oleate are not affected by adenosine). If this ratio played a role at some stage in the regulation of gluconeogenesis and urea synthesis it would explain inhibitions. It is also of interest that the mass-action ratio of the adenylate kinase system increases in the presence of adenosine (Table 3).

The changes in the redox state of ketone bodies in the presence of lactate and of the lactate dehydrogenase system also remain unexplained. It is relevant that another inhibitor of gluconeogenesis from lactate, quinolinate, changes the ketone-body ratio in the same direction as does adenosine (Williamson *et al.*, 1971).

Note Added in Proof (Received 24 October 1975)

Wilkening *et al.* (1975), in a paper published after the present paper was submitted, describe similar increases of adenine nucleotides on perfusion of the isolated rat liver with adenosine. However, they found no inhibition of gluconeogenesis from lactate. This difference may be due to experimental differences; they pre-perfused the liver with adenosine for 60 min before adding lactate.

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