THE EFFECTS OF GELATIN AND BOVINE SERUM ALBUMIN ON Ca^{2+} STIMULATION OF GLUCONEOGENESIS IN ISOLATED RAT HEPATOCYTES

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1. Introduction

Recently there has been some discussion on the relative merits of the use of gelatin and bovine serum albumin (BSA) for incubation of isolated liver cells. Binding of fatty acids to BSA, but not to gelatin, has been stated as the primary advantage of BSA over gelatin.

Ca^{2+} has been shown to stimulate gluconeogenesis in kidney [1], perfused liver [2] and isolated liver cells [3–5]. With isolated liver cells there are however large differences in magnitude of the effects of Ca^{2+} between the results of Siess and Wieland [3] and Zahlten et al. [5], and those obtained by Tolbert and Fain [4] and in this laboratory. The common difference in technique between these groups is the use of gelatin by the former and BSA by the latter groups.

Comparative studies on rates of gluconeogenesis from lactate in isolated liver cells in the presence of gelatin or BSA are reported. The differences in effects can be accounted for by the amounts of Ca^{2+} and NH_4^+ in the gelatin preparations.

2. Materials and methods

BSA was obtained from Armour Chemical Co. Ltd., Eastbourne, Sussex, and was treated to remove fatty acids before use [6]. Gelatin was obtained from three sources: Sigma (London) Chemical Co. Ltd., Difco Labs., Detroit, Michigan and Oxoid (London) Ltd. Lactate, glucose oxidase and peroxidase were obtained from Sigma. Collagenase (grade II), 2-oxoglutarate, NADH and glutamate dehydrogenase were obtained from Boehringer Corporation (London) Ltd.

Male Wistar rats (180–250 g) were starved for 48 h. before use. Isolated liver cells were obtained by the method of Krebs et al. [7] with the following modifications: (a) no hyaluronidase was used, (b) the in vitro incubation was omitted and (c) the medium was Ca^{2+}-free throughout the preparation. Incubations were performed at 37°C in 20 ml siliconised glass scintillation bottles. Each bottle contained approx. 5 mg dry weight of cells in 2 ml of Krebs–Henseleit saline [8] containing either 2% BSA or 1.5% gelatin and variable amounts of $CaCl_2$. Cells were preincubated for 40 mins before the addition of 10 mM lactate, and the rate of glucose production was measured over the period from 30 min to 90 min after the addition of substrate. Over this period the rate of gluconeogenesis was linear.

Ca^{2+} was measured by atomic absorption spectroscopy. Glucose [9] and NH_4^+ [10] were assayed by standard techniques.

3. Results

3.1. *Effect of Ca^{2+} on gluconeogenesis*

The dependence of the rate of glucose production from lactate on the Ca^{2+} concentration in medium containing 2% BSA is shown in fig.1. As can be seen the rate in the absence of added Ca^{2+} is only 14% of that with 2.5 mM Ca^{2+} (the Ca^{2+} concentration in full Krebs–Henseleit saline). In the presence of 1.5% gelatin the rate of gluconeogenesis in the absence of

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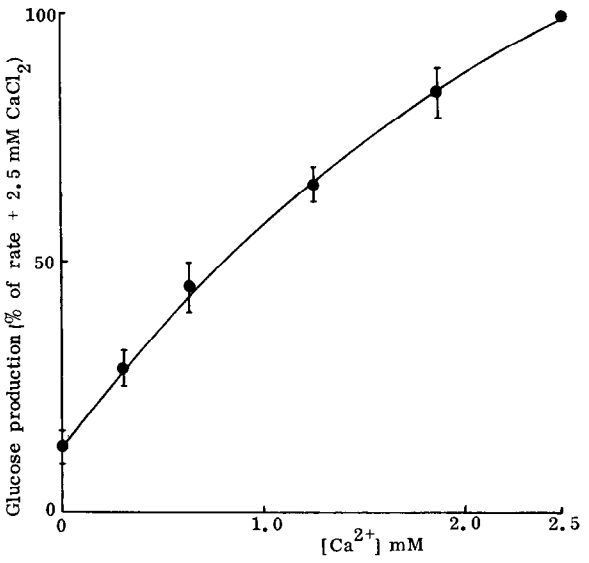

Fig.1. The effect of Ca^{2+} on gluconeogenesis from 10 mM lactate by isolated liver cells. Rates of glucose production (± S.E.M. of 4 experiments) expressed as percent of rate with 2.5 mM $CaCl_2$. Absolute rate with 2.5 mM $CaCl_2$ was 231 ± 28 nmol/mg dry wt./h. Cells (approx. 5 mg dry wt.) preincubated in Krebs–Henseleit saline containing 2% BSA for 40 min before addition of 10 mM lactate. Rate of glucose production measured from 30 min to 90 min after addition of substrate.

added Ca^{2+} was similar to that with 2% BSA and 2.5 mM Ca^{2+} (table 1). Addition of 2.5 mM Ca^{2+} to incubations containing 1.5% gelatin caused only a slight increase in the rate of glucose production (table 1). The addition of 2.5 mM EGTA in the absence of added Ca^{2+} decreased the rate of gluconeogenesis in all cases (table 1).

3.2. *Ca^{2+} and NH_4^+ contents of gelatin*

In view of the decrease in glucose production effected by EGTA, a specific chelator of Ca^{2+}, the Ca^{2+} contents of the various gelatin preparations was measured. Table 2 shows that although all three

Table 2
Ca^{2+} and NH_4^+ content of BSA and gelatin

	Ca^{2+}	NH_4^+
2% BSA	6 μM	15 μM
1.5% Oxoid gelatin	1.9 mM	0.40 mM
1.5% Sigma gelatin	0.43 mM	0.35 mM
1.5% Difco gelatin	0.20 mM	0.74 mM

Ca^{2+} and NH_4^+ concentrations measured in incubation medium containing either 2% BSA or 1.5% gelatin

Table 1
Effect of Ca^{2+} on gluconeogenesis

	No Ca^{2+}	2.5 mM Ca^{2+}	2.5 mM EGTA
2% BSA	31 ± 9 (5)	227 ± 17 (9)	12 ± 4 (3)
1.5% Oxoid gelatin	197 ± 16 (7)	231 ± 28 (4)	41 ± 6 (5)
1.5% Sigma gelatin	250 ± 27 (4)	291 ± 34 (3)	23 ± 4 (3)
1.5% Difco gelatin	223 ± 20 (3)	262 ± 33 (3)	39 ± 8 (3)

Cells (approx. 5 mg dry wt.) preincubated in Ca^{2+}-free Krebs–Henseleit saline with stated additions for 40 min before addition of 10 mM lactate. Rates of glucose production measured from 30 min to 90 min after of substrate. Rates expressed as nmoles glucose/mg dry wt./h ± S.E.M. for number of experiments in parenthesis.

preparations of gelatin contained significant amounts of Ca^{2+}, the Ca^{2+} content of both Difco and Sigma gelatin is lower than might be expected on the basis of the rates of glucose production. This indicated that there may possibly be some other activator in the gelatin preparations.

In the presence of BSA, if lactate is added without any preincubation there is a lag in glucose production before a linear rate is obtained [11]. This lag may be abolished by addition of NH_4^+ [11] which also has the effect of increasing the rate of gluconeogenesis [5,11]. In the presence of gelatin with no preincubation the lag is very small or even nonexistent. All three gelatin preparations tested were found to contain significant amounts of NH_4^+ (table 2).

4. Discussion

This study has brought to light an important problem in the use of gelatin in studies with isolated liver cells. Unlike the majority of chemicals used in research, which are of analytical reagent quality, the preparations of gelatin were very variable. All three preparations of gelatin contained Ca^{2+}, but only that obtained from Oxoid contained enough to explain the very high rates of glucose production on this basis alone. The discovery of NH_4^+ in the gelatin could account for the high rates of gluconeogenesis. These findings thus account for the fact that both Zahlten et al. [5] and Siess and Wieland [3] obtained high rates of gluconeogenesis in the absence of added Ca^{2+}, and that there was only a small increase on adding extra Ca^{2+}. As both NH_4^+ and Ca^{2+} show interactions with glucagon in its effect on gluconeogenesis [3,5] the existence of both these ions in gelatin means that it must be used with caution.

It was not possible to perform comprehensive tests for contamination of the gelatin, but in view of the existence of Ca^{2+} and NH_4^+ in such high concentrations, it is probable that there are other contaminants. If any free amino acids are present in the gelatin then these could affect the rates of gluconeogenesis by isolated liver cells [11].

It is possible to remove the small molecular weight contaminants by dialysis. However this poses a technical problem as below about 20°C (the exact temperature depends on the source of the gelatin) a 1.5% solution of gelatin forms a gel. In the gel state dialysis is prohibitively slow and so any dialysis must be performed at a temperature greater than 20°C.

A further problem associated with the use of gelatin is that, unlike BSA, gelatin does not precipitate in the presence of 2% perchloric acid. Thus any assay in which there is an interference by protein will be affected by gelatin despite the addition of perchloric acid.

In conclusion it appears that the use of BSA is preferable to gelatin for incubation of isolated liver cells. If gelatin must be used then the following points must be borne in mind: (a) gelatin does not bind fatty acids; (b) gelatin is not precipitated by perchloric acid; and (c) there are important impurities in gelatin which should be removed by dialysis.

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