Effect of Pretreatment of Pancreas Slices with n-Ethylmaleimide on Insulin Secretion *in vitro*.* (28202)

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It has been suggested that the primary mechanism of action of insulin may depend upon a reaction involving -SH groups on the cellular receptor and the S-S bridge of the peptide hormone(1,2,3,4). Although this hypothesis has not been confirmed, we were interested to know whether an agent blocking the -SH groups like n-Ethylmaleimide (EM) could produce an effect on insulin secretion *in vitro* when this substance was added to the buffer surrounding pancreas slices.

Material and methods. Small pieces of pancreas were obtained from ducks and rabbits. Pancreas slices from ducks were incubated in buffer (Krebs-Henseleit) containing EM 1×10^{-3} M during a period of 3 minutes. After washing, the pancreas slices were incubated for 15 minutes in the buffer containing glucose (3 mg/ml) or A.T.P. 1×10^{-3} M as stimulus for insulin secretion(5). Estimation of the buffer insulin was performed by the technique already described (6). When pieces of rabbit pancreas were used, after pretreatment with EM they were incubated for 3 minutes in a buffer containing A.T.P. 1×10^{-3} M. Insulin extraction of the buffer was performed according to the method of Grodsky and Tarver(7) and estimated by the technique of the epididymal fat tissue. In both experiments controls were slices or pieces of the same pancreas preincubated in buffer without EM and exposed to the same stimulus (glucose or A.T.P.).

The insulin effect was expressed as glucose uptake. Statistical analysis of the results was performed using the paired observations method(8).

Results and discussion. Pre-incubation of pancreas from ducks with EM induces a decrease in insulin secretion, using either glucose or A.T.P. as stimulus (Table I). Mirsky has demonstrated that EM does not prevent penetration of glucose in the cell. The same applies to the experiments performed with pancreas from rabbits where the differences obtained proved to be more significant. It seems from these experiments that EM has a limiting effect on release of insulin from the islets of Langerhans.

TABLE I. Effect of Glucose (3 mg/ml) and A.T.P. (1×10^{-3} M) on Insulin Secretion *in vitro* by Duck and Rabbit Pancreas Pre-Incubated With n-Ethylmaleimide (1×10^{-3} M).

Metabolites*		Glucose uptake (mean)		
Duck				
N-EM + glucose	(30)	.488	±.014†	P = .01
Glucose	(30)	.528		
N-EM + A.T.P.	(10)	.493	±.027†	P = .02
A.T.P.	(10)	.574		
Rabbit				
N-EM + A.T.P.	(20)	1.046	±.029†	P < .01
A.T.P.	(20)	1.309		

* Figures in parentheses are No. of experiments.
† Standard error of mean.

Summary. Insulin secretion as obtained from pancreas slices from ducks and rabbits after pre-incubation in a buffer containing n-Ethylmaleimide was stimulated with glucose and A.T.P. A remarkable diminution of the release of this hormone was observed with the slices previously incubated with EM.

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