Insulin-like Activity of a Dialyzable Factor in Human Blood Plasma *

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Human blood serum or plasma exerts an insulin-like activity shown by the stimulating effect on glucose uptake of the rat diaphragm *in vitro*. Based on this observation a method for determination of the insulin content in plasma or serum has been worked out by Groen *et al.*[1] and later by others. However, a definite proof that the effect of serum is due solely to the content of insulin is lacking. This can be questioned from the fact that wide variations in values obtained by different investigators have been reported, as well as from a theoretical point of view.

The present investigation has been undertaken to show if the insulin-like activity of plasma specifically can be related to the content of insulin or if other compounds contribute to this effect. The following experimental technique has been adopted. Blood is drawn into a syringe previously heparinized and plasma immediately separated by centrifugation. A sample of plasma was dialysed against Krebs Ringer solution for 12 h at +2 °C. The solution was buffered either with bicarbonate or with phosphate to pH 7.4 and glucose added to a final concentration of 140 mg per 100 ml. The effect of plasma and dialysate on glucose uptake of the rat diaphragm has been investigated by a procedure used for some years in our laboratory.

The dialysate from serum as well as from plasma stimulated glucose uptake in the isolated rat diaphragm. This was a significant and reproducible effect. As an average of 10 experiments 30 % stimulation was obtained. The stimulating effect was stable against heat. Thus, if the dialysate was kept at 80 °C for 20 min or boiled for 5 min the stimulating effect was increased by another 30 %. This indicated thermal inactivation of an inhibitory factor in the dialysate. The stimulating effect in the dialysate was not due to insulin. 0.1 unit crystalline insulin added to plasma did not influence the effect of the dialysate on glucose uptake of the rat diaphragm and insulin could be recovered in the plasma inside the bag after dialysis. — The insulin like effect of plasma was reduced after dialysis. After prolonged dialysis for 48 h even inhibition of the glucose uptake in rat diaphragm by the dialysed plasma has been observed.

So far, we have not been able to confirm that the insulin-like activity of plasma solely is due to the content of insulin. Of particular interest is that a dialysable factor from plasma or serum exerts insulin-like activity in the rat diaphragm method. Further studies on the nature of this factor is in progress.

* Supported by grants from *Eli Lilly and Co.*

1. Groen, J., Kamminga, C. E., Willebrands, A. F. and Blickman, J. R. *J. Clin. Invest.* **31** (1952) 97.

The Interaction of Cysteamine and Cysteine with various Carbonyl Compounds *

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Numerous important metabolites and several therapeutic agents possess reactive carbonyl groups. A study of the interaction of thiols with carbonyl compounds has therefore been undertaken.

In the present paper data on the interaction of the 1-mercapto-2-amines cysteamine and cysteine with several carbonyl compounds, will be reported. The procedure previously described for the analysis of thiol-disulphide interactions [1,2], was found suitable for this purpose. The sulphur labeled thiols were incubated with the carbonyl compound at 37 °C and the desired pH. After acidification the labeled products were separated from the reactants by paper electrophoresis in phthalate buffer at pH 2.

The reaction mechanism has been studied in some detail in the system cysteamine and glyceraldehyde. It was observed that at pH 6.8—7.4 the rate of formation of the labeled product followed the kinetics of a bimolecular one-way reaction of the type $A+B \rightarrow C$. No evidence for the formation of dithials was

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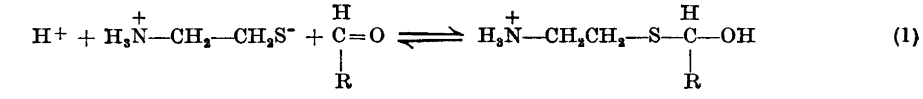
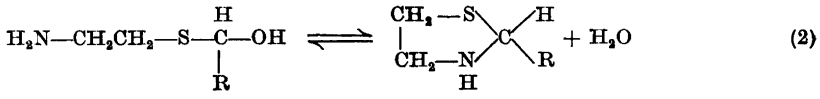
obtained, even when a large excess of cysteamine was used. Over a wide pH range (1.4—8.3) the observed reaction rate paralleled the concentration of the ionized thiol ($pK_{SH}=8.6$), which constitutes evidence that in this reaction thiol is indeed the reactive molecular species. It also demonstrates that, in the pH range studied, no marked proton activation of the carbonyl group takes place. At pH 7.4 and 37 °C the radioactivity of the labeled product did not decrease when an excess of non-labeled cysteamine was added.

On the basis of the above data the following reaction mechanism is suggested:



(1)



(2)

Since the formation of cyanohydrins, acetals and bisulphite addition products are known to be reversible [3], reaction 1 would similarly be expected to be reversible. The fact that the formation of the product appears as a one-way reaction, can be explained by assuming that reaction 2 is faster than reaction 1 and is displaced to the right.

The rate of interaction of cysteamine with cortisone was found to be negligible at pH 7.4 and 37 °C. However, significant reaction rates were found with glyceraldehyde, methylglyoxal [4], dihydroxyacetone and α-ketoglutaric acid. The rate constants

$$k = \frac{[\text{Product}]}{[RS^-]\,[R'\text{—}CO\text{—}R'']}\ \text{min}^{-1} \times \text{M}^{-1} \times (10^3)\text{l}$$

were 2.5, 1.2, 0.2 and 0.01 respectively.

Since streptomycin is known to be inactivated by cysteamine and cysteine [5], it is of interest that cysteine was found to react stoichiometrically with streptomycin at a fairly rapid rate, whereas no interaction was observed with dihydrostreptomycin. This latter finding indicates that the reaction takes place at the carbonyl group of streptomycin.

1. Eldjarn, L. and Pihl, A. *Acta Chem. Scand.* **10** (1956) 1054.
2. Eldjarn, L. and Pihl, A. *J. Biol. Chem.* **225** (1957) 499.
3. Bartlett, P. D. in *Organic Chemistry* **3**, John Wiley & Sons, Inc. New York 1953, p. 1.
4. Franzen, V. *Angew. Chem.* **11** (1956) 381.
5. Denkelwater, R., Cook, M. A. and Tishler, M. *Science* **102** (1945) 12.

The Isolation of Prostaglandin

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The presence of a smooth muscle stimulating factor in sperm and in extracts of the accessory genital glands of man and certain animals was demonstrated by Goldblatt [1] and von Euler [2]. A concentrate of the factor from sheep prostate gland was prepared by von Euler [3] who studied some of its chemical properties.

Some preliminary data on the further purification of this concentrate have been published [4].

We have succeeded in isolating one "prostaglandin" factor (PGF) in crystalline form The. compound (102°—103°) is an unsaturated hydroxyacid, that does not contain nitrogen. A good response is obtained on a rabbit's duodenum at a concentration of $\sim 5 \times 10^{-9}$ g per ml.

At least one other active acidic factor is present in sheep prostate glands.

The isolation procedure and some of the properties of the pure compound will be discussed.

1. Goldblatt, M. W. *Chemistry & Industry* **52** (1933) 1056.
2. von Euler, U. S. *Arch. exptl. Pathol. Pharmakol* **175** (1934) 78.
3. von Euler, U. S. *Scand. Arch. Physiol.* **81** (1939) 65.
4. Bergström, S. *Nord. Med.* **42** (1949) 1465.