# THE INFLUENCE OF VARIOUS HORMONES ON THE UTILIZATION OF GLUCOSE*

by

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## INTRODUCTION

In a previous paper[1] some evidence was provided that the glucose utilization of rat diaphragm, as measured by the anaerobic glycolysis, was impaired in animals which had been injected with adrenaline. It was suggested that this decrease in the utilization of glucose accounted for part of the hyperglycaemia observed after administration of adrenaline. Since adrenaline when added *in vitro* had never any effect on the glucose utilization of diaphragm, it was suggested that the adrenaline action might be indirect. Such an indirect effect could be conceived in a variety of ways.

A possible mechanism of indirect action would be the transformation of adrenaline somewhere in the body (not necessarily at the site of action) into an active compound.

Perhaps the most likely mechanism in view of present knowledge of hormonal balances would be the action of adrenaline through or in co-operation with other hormones. Adrenaline might inhibit or stimulate the release of hormones from other endocrine glands; alternatively it might make possible the peripheral action of such hormones, released in unchanged quantities.

In this paper some of the possibilities of the second type of indirect action of adrenaline (through mediation of other hormones) were investigated. The endocrine glands which at first sight are likely to play a role (apart from the adrenal medulla) in the process of glucose utilization are:

a. Hypophysis
b. Adrenal Cortex
c. Pancreas
d. Thyroid

The increased general metabolism after adrenaline (calorigenic effect) in the presence of a decreased glucose oxidation makes the mediation of the thyroid (which is known to give a generally increased metabolism including carbohydrate oxidation) unlikely.

A decrease of insulin production by the pancreas or an impaired peripheral insulin action under influence of adrenaline could account for the observed fall in glucose utilization; only, no such effect of adrenaline on the pancreas or on insulin has ever been

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shown. On the contrary, increased production of insulin is often regarded as a sequel of the administration of adrenaline.

Although the possibility of an effect of adrenaline in connection with the pancreas or the thyroid gland can by no means be regarded as excluded, research into the possibilities *a* and *b* was considered to be the more promising initial approach.

Evidence exists that the hypophysis and the adrenal cortex are involved in the utilization of glucose. J. A. RUSSELL[2, 3, 4, 5, 6] in particular has been able to demonstrate an apparent rapid rate of disappearance of carbohydrate in the absence of the hypophysis and its prevention by APE (anterior pituitary extract). She showed that, apart from an effect of the hypophysis on gluconeogenesis, an influence of the hypophysis on carbohydrate oxidation must be postulated. Other authors arrived at the same conclusions[7, 8, 9, 10].

Since the rapid rate of disappearance of blood sugar noted after hypophysectomy as compared to unoperated controls persists in the absence of the liver, the prime site of glucose production, it seems reasonable to assume that peripheral utilization of blood sugar proceeds at an accelerated rate in the absence of the hypophysis.

LONG *et al.*[11, 12] and RUSSELL[13, 14] showed that apart from APE also adrenocortical hormones have an inhibitory influence on the utilization of glucose.

COLOWICK, CORI *et al.*[15*] demonstrated that hypophysis extracts are able to inhibit the hexokinase action of muscle extract *in vitro*. This effect is enhanced by adrenal cortical extract (ACH) and reversed by insulin. This suggests that the hypophysis either alone or in collaboration with ACH could exert an inhibition on glucose utilization in the sense used in this paper. ACH could also be effective alone provided that there is already some intrinsic hypophysis factor present in the muscle preparation at the moment ACH is added.

About relationships between adrenaline on the one hand and the hypophysis and adrenal cortex on the other hand little information is available. An important contribution in this respect is the work of VOGT[16]. She showed that a long lasting output of adrenal cortical hormones followed an intravenous infusion of adrenaline in dogs.

The following experiments were carried out in order to examine the possibility that the effets of adrenaline on glucose utilization by diaphragm described in a previous paper[1] were mediated through pituitary and adrenal cortical hormones.

*Experimental methods*

The diaphragms were prepared and the anaerobic glycolysis estimated as described previously[1].

Hypophysectomies were performed according to FREUD's modification of SMITH's method[17]. The success of the hypophysectomy was assessed by local inspection of the site and by microscopic sections of the adrenal cortex (appearance of sudanophobic zone).

The adrenals were taken out extraperitoneally from the back using a small median skin incision.

## EXPERIMENTAL RESULTS

### A. INFLUENCE OF HORMONES ON THE GLYCOLYSIS OF RAT DIAPHRAGM

The different hormones to be tested were incubated together with diaphragm from normal rats; the usual constituents of the medium and the estimation and evaluation

* The endocrine interrelationships described by COLOWICK, CORI *et al.* will in future be referred to as the "CORI mechanism".

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of the $Q^{N_2}_{CO_2}$ were carried out exactly in the same way as has been described previously, using the rate of glycolysis occurring in the period elapsing from 20 to 50 minutes after the beginning of the incubation as basis for the calculation of the $Q^{N_2}_{CO_2}$. It was not considered necessary to accompany every experiment with a blank control (glycolysis in the absence of glucose), because blank glycolysis is low during this period and because it could be established that the rate of blank glycolysis was not significantly influenced by the presence of hormones.

Experiments involving hormones were of course accompanied by a control in the presence of glucose alone and with no hormones added. For every experiment the diaphragms of 4 untreated 24 hours starved rats from the same litter were used. These diaphragms were pooled and divided into four portions for different manometric estimations. The portions were made up from approximately equal contributions from each of the four diaphragms and were therefore comparable.

1. *Anterior pituitary extract (APE)*

The APE used for these experiments was a diabetogenic hypophysis extract kindly provided by Professor F. G. YOUNG. A fraction purified by precipitation at $p_H$ 6 was used. The results are shown in Table I. From a comparison of columns 3 and 4 it appears

TABLE I

GLYCOLYSIS OF RAT DIAPHRAGM UNDER INFLUENCE OF VARIOUS HORMONES

| Expt | Controls | | APE 0.2 ml | ACE 0.2 ml | Insulin 1‰ 0.2 ml | APE + ACE | APE + ACE + Insulin | ACE + Insulin |
|---|---|---|---|---|---|---|---|---|
| | Blank | + Glucose | | | | | | |
| 1 | 1.20 | 2.48 | 2.64 | — | — | 1.44 | — | — |
| 2 | 1.00 | 1.56 | — | 1.48 | — | 1.26 | — | — |
| 3 | — | 2.00 | — | — | 2.48 | 1.72 | 1.88 | — |
| 4 | — | 3.54 | — | — | 3.26 | 2.08 | 1.72 | — |
| 5 | — | 1.70 | 1.86 | 1.40 | 2.48 | — | — | — |
| 6 | — | 1.96 | — | — | 2.60 | — | — | — |
| 7 | — | 5.18 | — | — | 4.24 | 2.78 | 3.86 | — |
| 8 | — | 2.10 | 1.96 | — | 3.54 | — | — | — |
| 9 | — | 1.82 | — | 1.44 | 2.38 | — | — | — |
| 10 | — | 1.90 | 1.76 | — | 2.18 | — | — | — |
| 11 | — | 1.94 | — | — | 2.26 | 1.22 | 1.42 | — |
| 12 | — | 1.72 | 2.28 | 1.40 | — | 1.74 | — | — |
| 13 | 0.76 | 2.70 | — | 1.8 | — | 1.4 | — | — |
| 14 | — | 1.88 | — | 1.4 | 2.4 | — | — | — |
| 15 | — | 3.2 | — | 1.32 | 2.08 | — | — | 2.28 |
| 16 | — | 1.44 | — | 1.44 | 2.76 | — | — | 1.84 |
| 17 | — | 2.14 | — | 1.32 | 2.28 | — | — | 1.32 |
| 18* | — | 2.28 | 1.56 | 1.80 | — | 1.72 | — | — |

* Crude diabetogenic APE was used.
The figures express $Q^{N_2}_{CO_2}$.
The experiments were carried out in BARCROFT manometers at 38° C.
Volume 3 ml. Medium: bicarb. KREBS HENSELEIT RINGER. Glucose 0.2%.
Gas phase $N_2/CO_2$ 5%.
The APE used was a $p_H$ 6 insoluble dialysed hypophysis extract.
The ACE used was eschatin (without preservative).
The insulin used was either crystalline BOOTS' insulin or BLATHERWICK powder.
The hormones were mixed with the medium at the beginning of the incubation period.
Mean difference between insulin treated diaphragm and control (13 expts) + 0.30 ± 0.20 P = 0.15.
Mean difference between ACE treated diaphragm and control (10 expts) −0.55 ± 0.16 P = < 0.01.

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that no convincing inhibition of glycolysis takes place in the presence of APE as compared to the glycolysis in the control with the exception of experiment 18 where crude extract was used instead of the $p_H$ 6 insoluble fraction.

2. *Adrenal cortical extract (ACE)*

Eschatin prepared without preservative and kindly provided by MESSRS. PARKE DAVIS was used in these experiments. It gave a marked inhibition of the glycolysis of rat diaphragm in 8 out of 10 experiments (Table I).

3. *Insulin*

The insulin used was either crystalline BOOTS' insulin or an insulin sample provided by BOOTS with a low zinc content (0.1%). In other experiments BLATHERWICK powder was used. No difference in action between these preparations was observed. Before use the insulin was twice precipitated iso-electrically. Before each experiment it was dissolved in phosphate (M/100) saline buffer. A study of Table I reveals that out of 13 experiments insulin activates the glycolysis 10 times. In the remaining 3 experiments there is a control value higher than 2.8. In the 10 experiments where insulin activates, the control value is invariably lower than 2.8. It therefore appears that insulin has an activating effect provided that the control is not too high. It is likely that the intrinsic hormonal equilibrium determines what effect the added insulin is going to produce. No explanation is offered for the occasional *inhibition* observed with insulin (experiments 4 and 7, column 6).

These experiments show that decrease of glucose utilization may be brought about through mediation of the adrenal cortex. They also show that insulin has an activating effect on glucose utilization. Under the experimental conditions diabetogenic hypophysis extract has however no effect on glucose utilization. Of course, these observations cannot be considered as evidence in favour of or against a CORI mechanism. It is possible that ACE can only exert its inhibiting effect on account of intrinsic APE present in the diaphragm at the beginning of the experiment. The failure of APE to show an effect of its own may be caused by the presence of already optimal intrinsic quantities before APE is added. Other possibilities are inability of the diabetogenic extract used to penetrate into the diaphragm or to exert a hexokinase inhibiting activity anyway. In this connection it may be mentioned that CORI was unable to show hexokinase inhibition using diabetogenic extracts.

The combinations used were chosen in the hope of being able to make manifest a possible CORI mechanism (inhibition of glucose utilization by APE, enhanced by ACE and reversed by insulin).

Table I shows further that insulin usually increases glycolysis in the presence or absence of other hormones (ACE alone (column 9) or ACE plus APE (column 8)).

## B. THE GLYCOLYSIS IN DIAPHGARM OF HYPOPHYSECTOMIZED AND ADRENALECTOMIZED ANIMALS

The glycolysis of diaphragm in hypophysectomized and adrenalectomized rats after adrenaline was determined and calculated exactly in the same way as has been described before. Again the animals were starved for 20–24 hours before the experiments. The adrenalectomized animals were kept in good condition by giving them 1% NaCl

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solution ad libitum. The experiments on the hypophysectomized animals were carried out 7–10, those on the adrenalectomized 3–4 days after the operation. Adrenaline was injected subcutaneously in a dose of 1 mg/kg. The results are presented in Table II and Table III. Every figure represents the mean of the glycolysis of two different diaphragms.

The tables show that in hypophysectomized animals adrenaline is no longer able to produce its inhibiting effect on the glucose utilization. This indicates that the presence of the hypophysis is necessary for the adrenaline effect to become manifest. Possibly

TABLE II

DIAPHRAGM GLYCOLYSIS IN HYPOPHYSECTOMIZED RATS

| Expts | Hypophysectomized controls | | | Hypophysectomized rats injected with adrenaline 1 mg/kg subcut | | |
|---|---|---|---|---|---|---|
| | Blanks $Q_{CO_2}^{N_2}$ | In presence of 0.2% glucose $Q_{CO_2}^{N_2}$ | Difference due to glucose utilization | Blanks $Q_{CO_2}^{N_2}$ | In presence of 0.2% glucose $Q_{CO_2}^{N_2}$ | Difference due to glucose utilization |
| 1 | — | 1.54 | — | 0.54 | 2.92 | — |
| 2 | 0.46 | 2.20 | — | 1.24 | 3.04 | — |
| 3 | 1.16 | 2.52 | — | 1.16 | 2.62 | — |
| 4 | 2.38 | 5.24 | — | 1.20 | 2.44 | — |
| 5 | 0.74 | 2.02 | — | 0.74 | 2.04 | — |
| 6 | 0.64 | 1.68 | — | — | 2.32 | — |
| 7 | 1.02 | 1.94 | — | 0.96 | 1.90 | — |
| 8 | — | 0.76 | — | — | 1.72 | — |
| 9 | — | 1.4 | — | — | 2.56 | — |
| 10 | — | 1.22 | — | — | 10.4 | — |
| 11 | — | 2.16 | — | — | 2.06 | — |
| 12 | 1.04 | 3.04 | — | 0.5 | 1.56 | — |
| 13 | 0.8 | 2.78 | — | 1.08 | 3.88 | — |
| Mean | 1.04 | 2.20 | 1.16 | 0.92 | 2.32 | 1.40 |

Every figure represents the mean $Q_{CO_2}^{N_2}$ of two diaphragms.
* The conditions were the same as in previous experiments.

TABLE III

DIAPHRAGM GLYCOLYSIS IN ADRENALECTOMIZED RATS

| Expts | Adrenalectomized control rats | | | Adrenalectomized rats after adrenaline 1 mg/kg subcut. | | |
|---|---|---|---|---|---|---|
| | Blanks $Q_{CO_2}^{N_2}$ | In presence of glucose $Q_{CO_2}^{N_2}$ | Difference due to glucose utilization | Blanks $Q_{CO_2}^{N_2}$ | In presence of glucose $Q_{CO_2}^{N_2}$ | Difference due to glucose utilization |
| 1 | 1.54 | 2.52 | — | 1.16 | 3.06 | — |
| 2 | 1.38 | 3.12 | — | 1.04 | 1.92 | — |
| 3 | 1.56 | 4.6 | — | 0.84 | 2.6 | — |
| 4 | 1.46 | 2.62 | — | 1.26 | 2.16 | — |
| 5 | 1.32 | 5.12 | — | 1.16 | 2.2 | — |
| Mean | 1.44 | 3.60 | 2.16 | 1.10 | 2.38 | 1.28 |

Every figure represents the mean $Q_{CO_2}^{N_2}$ of two diaphragms.
* The conditions were the same as in previous experiments.

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the hypophysis exerts some influence on the absorption of adrenaline from the site of injection as suggested by CORI[18], but a fair amount of adrenaline must be absorbed in hypophysectomized animals, as is testified by clinical symptoms not noticeably differing in intensity from those in normal animals. It is therefore unlikely that the *complete* failure of adrenaline to show its usual effect upon glycolysis in hypophysectomized animals can be attributed to poor absorption from the site of injection. A more likely possibility is the absence in hypophysectomized animals of the release of a hexokinase inhibiting substance from the hypophysis under influence of adrenaline.

In adrenalectomized animals the inhibiting effect of adrenaline on glycolysis is maintained unimpaired. This makes it unlikely that adrenaline exerts its effect on glucose utilization either through direct stimulation of the adrenal cortex (as described by VOGT) or indirectly over the pituitary corticotrophic hormone.

## DISCUSSION

The absence of an adrenaline response in hypophysectomized animals suggests that the hypophysis is involved is some way in the indirect inhibitory adrenaline action on peripheral glucose utilization. This adrenaline effect could be the release of a pituitary factor capable of inhibiting the utilization of glucose.

The pituitary factor most likely to be responsible for such an effect would be either YOUNG's diabetogenic factor[28] or the hexokinase inhibiting factor of CORI *et al.*[19]. These factors are probably not identical. The diabetogenic factor has no direct inhibiting effect on hexokinase *in vitro*, but experiments by SMITH AND YOUNG[20] indicate that *in vivo* administration of the diabetogenic factor may lead to inhibition of hexokinase, possibly through production of the CORI factor.

The diabetogenic factor has no effect on diaphragm glycolysis when added *in vitro* which may be due either to poor penetration or to absence under our experimental conditions of the above mentioned *in vivo* mechanism necessary to produce hexokinase inhibition after the administration of diabetogenic factor. These experiments confirm the results of RIESSER[21] and of OTTAWAY AND SMITH[29], who found that APE per se does not give an outspoken inhibition of glucose utilization in extracts and diaphragm. The latter authors did find that APE abolishes the increased glucose uptake in diaphragm from a medium containing insulin (GEMMILL AND HAMMER)[22] and STADIE AND ZAPP[23]. In our experiments insulin partly reverses the inhibition by APE and ACE. Whether or not this insulin effect is due to a reversal of inhibitive action (of added or intrinsic hormones) or to an independent stimulating effect, cannot be deduced from these experiments. The insulin reversal in presence of added ACE (or ACE plus APE) never amounts to more than a partial restoration of the original activity.

The indirect adrenaline inhibition is not influenced by the presence of the adrenals and is therefore independent of ACE.

It is interesting to note that in adrenalectomized animals the glycolysis is high compared with the controls. This is a confirmation of the reversed effect (namely the inhibition of glycolysis by eschatin) observed in our experiments and of the results of KRAHL AND CORI[24]. It appears that the adrenal cortical hormones although not mediating the action of injected adrenaline, make an important contribution in the establishment of the rate glucose utilization in normal animals.

Increased stability of liver glycogen as regards its breakdown to blood glucose

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under the influence of adrenaline in the absence of the hypophysis was reported by CRANDALL AND CHERRY[25] and DE BODO *et al.*[26, 27], who found that after adrenaline the output of glucose from the liver of a hypophysectomized dog averages less than 50% that of normal animals.

If an indirect inhibiting effect of adrenaline on glucose utilization (hexokinase), depending on the presence of the hypophysis, is postulated, then the impaired response to adrenaline in hypophysectomized animals may in my opinion be attributed to the general absence of this hexokinase inhibiting effect in hypophysectomized animals. It does not seem necessary to postulate a second mechanism, the increased glycogen stability. The so-called "stability of liver glycogen" may be interpreted as an increase of the "utilization" of glucose by the liver (in the sense defined of glucose uptake from the blood) because "decreased liver glucose output" as observed by CRANDALL AND CHERRY may be just an interpretation of increased glucose intake in hypophysectomized animals whose liver hexokinase (like the peripheral hexokinase) is no longer exposed to the inhibiting influence of the hypophysis.


## SUMMARY

1. Diabetogenic pituitary extracts do not inhibit the glycolysis of diaphragm under the conditions of the experiments described.

2. Insulin increases, eschatin inhibits the glycolysis of normal diaphragm. These effects may be due to the presence of intrinsic CORI factor in diaphragm.

3. Hypophysectomized animals do not show the inhibition of diaphragm glycolysis usually observed in animals treated with adrenaline indicating that the indirect adrenaline inhibition of diaphragm glycolysis is probably mediated by the hypophysis.

4. The indirect adrenaline inhibition is not influenced by the presence of the adrenals and therefore independent of adrenal cortical hormones.

## RÉSUMÉ

1. Des extraits diabétogènes de la glande pituitaire n'inhibent pas la glycolyse du diaphragme dans les conditions expérimentales décrites.

6. L'insuline augmente la glycolyse du diaphragme normal, tandis que l'"eschatine" l'inhibe. Ces effets sont peut-être dus à la présence du facteur intrinsèque de CORI dans le diaphragme.

3. Des animaux ayant subi l'ablation de l'hypophyse ne présentent pas l'inhibition de la glycolyse du diaphragme généralement observée sur les animaux traités à l'adrénaline; cela indique que l'inhibition de la glycolyse du diaphragme par l'adrénaline est indirecte, et s'effectue probablement par l'intermédiaire de l'hypophyse.

4. L'inhibition indirecte par l'adrénaline n'est pas influencée par la présence des glandes surrénales, et par conséquent indépendante des hormones cortico-surrénales.

## ZUSAMMENFASSUNG

1. Diabeterzeugende Hypophysenextrakte hemmen die Glykolyse des Zwerchfells unter den Bedingungen der beschriebenen Versuche nicht.

2. Insulin fördert, "Eschatin" hemmt die Glykolyse des normalen Zwerchfells. Diese Wirkungen beruhen möglicherweise auf der Gegenwart des innerlichen Cori-Faktors im Zwerchfell.

3. Hypophysenlose Tiere zeigen nicht die Hemmung der Zwerchfell-Glykolyse die gewöhnlich bei mit Adrenalin behandelten Tieren beobachtet wird; dadurch wird angezeigt, dass diese Adrenalin-Hemmung der Zwerchfell-Glykolyse eine indirekte ist, wobei die Hypophyse wahrscheinlich eine Zwischenrolle spielt.

4. Die indirekte Adrenalin-Hemmung wird durch die Gegenwart der Nebennieren nicht beeinflusst, und ist folglich unabhängig von den Nebennierenrinden-Hormonen.


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