# The Effects of Phenformin on the Isolated Rat Diaphragm

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Many of the effects of DBI, added to tissues in vitro, are now well documented. It can cause a marked increase in both the glucose uptake and the glycogen breakdown of the diaphragm[1] and in lactate production by this tissue.[2] Oxygen utilization of whole tissues and of homogenates is reduced.[3,4] We have repeated and extended some of these observations, and in addition, we have measured phosphate output by the isolated rat diaphragm under the influence of DBI. In an effort to gain more information on the mechanism of action of DBI, we have also measured the influence of this drug on the volume of distribution of certain pentoses. The results to be described give only a portion of our experimental data. More complete details will be reported elsewhere.

## EXPERIMENTAL

Normal (or alloxan diabetic) rats weighing 200 to 250 gm. were sacrificed by stunning. The diaphragm was then quickly removed and was trimmed and cut into two more or less equal portions—the hemidiaphragms. A small portion of each hemidiaphragm was cut off for a determination of the initial glycogen level.[5] The remaining two pieces were lightly blotted, weighed and added to the appropriate incubation medium. A modified Krebs-Henseleit medium was used, with the phosphate salt omitted. Five milliliters of the medium were placed in 25-ml. flasks, which were gassed with 95 per cent $O_2$-5 per cent $CO_2$ mixture after the tissues were added. In some of the flasks, DBI had been added to give a final concentration of 1.0 mg. per ml. Glucose or fructose was present in a concentration of 200 mg. per 100 ml. Glucose was determined by the Somogyi-Nelson method[6] or by a fermentation method, using glucose oxidase (Sigma). Inorganic phosphate was determined by the method of Fiske and SubbaRow,[7] lactate by the method of Barker and Summerson.[8]

In the experiments in which pentose spaces were measured, the intact diaphragm preparation of Kipnis and Cori[9] was used. Tissues were incubated for one hour at 37° C. in a Krebs-Henseleit medium which contained 400 mg. per cent pentose. Following the incubation, the diaphragm was removed and trimmed and a small portion was used for a determination of the water content. The remaining tissue was weighed, placed in boiling water and extracted for ten minutes. Aliquots of the resulting solution were used for the required analytical determinations. Pentoses were determined by the method of Roe.[10]

## RESULTS

Table 1 shows the results of experiments in which glucose or fructose uptakes were measured, along with changes in glycogen levels (initial glycogen level less

TABLE 1

Effect of DBI in vitro

| Animal | Normal | | Diabetic | | Normal | |
|---|---|---|---|---|---|---|
| Monosaccharide in medium | Glucose | | Glucose | | Fructose | |
| Monosaccharide uptake (mg./gm.) | | N | | N | | N |
| Control | 3.60 | 10 | 3.64 | 5 | 1.58 | 4 |
| DBI | 4.70 | 10 | 6.17 | 5 | 2.50 | 4 |
| Diff. | 1.10 | | 2.53 | | 0.92 | |
| P | <0.01 | | >0.05 | | <0.01 | |
| Glycogen change (mg./gm.) | | | | | | |
| Control | 0.52 | 6 | 1.20 | 4 | —1.30 | 4 |
| DBI | —1.00 | 6 | —0.82 | 4 | —1.85 | 4 |
| Diff. | —1.52 | | —2.02 | | —0.55 | |
| P | <0.01 | | <0.01 | | <0.01 | |
| Lactate output (mg./gm.) | | | | | | |
| Control | 1.83 | 6 | 2.78 | 4 | — | |
| DBI | 3.49* | 6 | 3.22 | 4 | — | |
| Diff. | 1.66 | | 0.44 | | — | |
| P | <0.05 | | >0.05 | | — | |
| Phosphate output (mg./gm.) | | | | | | |
| Control | 0.55 | 6 | 0.45 | 3 | 0.44 | 4 |
| DBI | 0.63 | 6 | 0.73 | 5 | 0.65 | 4 |
| Diff. | 0.08 | | 0.28 | | 0.19 | |
| P | >0.05 | | <0.025 | | >0.05 | |

*DBI conc. 0.5 mg./ml. for this particular experiment. In all other experiments the DBI concentration was 1 mg./ml.
N = Number of experiments.

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glycogen level after incubation). Values for lactate output and phosphate output are also given. DBI exerted a marked stimulating effect upon glucose and fructose uptake in tissues from normal animals; it had a similar effect on glucose uptake on tissues from diabetic animals. Glycogen breakdown was greatly increased, and there was an increase in lactic acid production. The increase in phosphate liberation was not quite so pronounced, but is nevertheless significant. Under similar experimental conditions, insulin decreased phosphate loss to the medium. The source of the phosphate may be rather important. It must be either phosphate esters such as the $C_6$ or $C_3$ compounds, or it may come from compounds with a high energy bond. If the source is one of the esters of the Embden-Meyerhof chain, then it seems likely that the production of lactic acid would be interfered with, and there would be a reduced lactate output, rather than an increased output. No direct measurements of ATP, ADP or CP were made, but these compounds would seem to be likely sources of the phosphate.

The results of an experiment similar to the well-known one of Park et al.[11] in which the tissues are incubated in a medium containing an extremely high concentration of glucose are shown in table 2. The figures suggest that insulin increases the intracellular glucose, but the increase is not statistically significant. However, since the experiment was essentially a repetition of the work of Park,[11] who found a significant increase in intracellular glucose, the action of insulin in these experiments appeared to be confirmed. DBI, on the other hand, caused a drop in intracellular glucose. This gives further confirmation to the idea that DBI increases the rate of glucose breakdown.

The effect of DBI, and of some other substances upon the value of distribution of some different pentoses is shown in table 3. It can be seen that DBI, in contrast to insulin or dinitrophenol, does not increase the pentose space. The water content and the insulin space of the diaphragm are unaltered by the drug (table 4). The failure of DBI to affect these values shows that any increase in sugar uptake (on the assumption that glucose and these pentoses are transported by a similar mechanism) cannot be ascribed simply to an increase in extracellular or intracellular fluid. Its effect must therefore be on one of the metabolic processes of the cell. Other workers have shown that it interferes with oxidative processes,[3,4] and it has been amply demonstrated by Randle[12] and others that anaerobiosis, or the presence of an inhibitor of aerobic metabolism may, in suitable media, stimulate glucose uptake or glycogen breakdown. The mode of action of DBI may be related to this.

TABLE 2

Effect of DBI on free glucose content of diaphragm*

| Control | Insulin | N | |
|---|---|---|---|
| 5.81 ±0.31 | 6.21 ±0.03 | 3 | No significant difference on small number of experiments. |
| Control | DBI | | |
| 6.22 ±0.50 | 5.20 ±0.48 | 7 | P 0.025 |

*Diaphragms incubated in heparinized dog plasma with glucose added to give a final concentration of 2,000 mg. per cent.

All values expressed as mg./gm. tissue, means ± standard deviation.

TABLE 3

Volume of distribution of pentoses (as per cent of wet weight, means ± standard deviation)

| | D-xylose | N | L-arabinose | N | D-arabinose | N | D-xylose (phosphate buffer) | N |
|---|---|---|---|---|---|---|---|---|
| Control | 53.7 ±3.5 | 5 | 42.3 ±1.5 | 4 | 40.8 ±5.0 | 4 | 46.4 ±2.6 | 5 |
| DBI | 53.4 ±1.8 | 5 | 48.4 ±4.3 | 4 | — | | 45.0 ±7.8 | 4 |
| Insulin | 74.3 ±1.5 | 4 | — | | 66.0 ±8.1 | 4 | 76.9 ±3.0 | 4 |
| Dinitrophenol (2.5 × $10^{-3}$m) | 69.5 ±4.9 | 7 | — | | — | | 66.4 ±4.9 | 5 |

N = Number of experiments.

TABLE 4

Effect of DBI on insulin space of water content

| | Insulin space (per cent wet weight) | Water content (per cent wet weight) | N |
|---|---|---|---|
| Control | 22.5 ±1.4 | 76.5 ± .7 | 8 |
| DBI | 24.8 ±3.1 | 76.5 ±0.7 | 8 |
| Insulin | 22.5 ±0.8 | 75.5 ±0.9 | 4 |
| Dinitrophenol (2.5 × $10^{-5}$m) | 22.8 ±2.7 | 80.5 ±0.6 | 8 |

N = Number of experiments. All values are mean values, ± standard deviation.

The effect of DBI again raises the question of the fundamental mechanisms which influence glucose uptake of the cell. Certainly permeability and glucose uptake are related, but the fact that conditions can be obtained, as with DBI, whereby there is an increased glucose uptake without the attendant increase in permeability, strongly suggests that this glucose uptake is influenced by many factors, of which the permeability of the cell is only one.

SUMMARY

Phenformin in vitro causes an increase in glucose uptake, glycogen breakdown, lactate output and output of inorganic phosphate of the isolated rat diaphragm.

Phenformin does not cause an increase in pentose space, insulin space, or water content of the intact isolated diaphragm.

SUMMARIO IN INTERLINGUA

*Le Effectos de Phenformina Super le Metabolismo del Isolate Diaphragma del Ratto*

In le isolate diaphragma del ratto, phenformin causa in vitro un augmento del fixation de glucosa, del decomposition de glycogeno, del rendimento de lactato, e del rendimento de phosphato inorganic.

In le intacte isolate diaphragma del ratto, phenformin non causa un augmento del spatio de pentosa, del spatio de insulina, o del contento de aqua.

REFERENCES

[1] Williams, R. H., Tyberghein, J. M., Hyde, P. M., and Nielsen, R. L.: Studies related to the hypoglycemic action of phenethyldiguanide. Metabolism 6:311-19, 1957.

[2] Williams, R. H., Tanner, D. C., and Odell, W. D.: Hypoglycemic action of phenethyl-, amyl-, and isoamyldiguanide. Diabetes 7:87-92, 1958.

[3] Wick, A. N., Larson, E. R., and Serif, G. S.: The site of action of phenethylbiguanide, a hypoglycemic compound. J. Biol. Chem. *233*:296-98, 1958.

[4] Steiner, D. F., and Williams, R. H.: Respiratory inhibition by biguanides and decamethyldiguanidine. Biochimica et Biophysica Acta 30:329-40, 1958.

[5] Good, C. A., Kramer, M., and Somogyi, M.: The determination of glycogen. J. Biol. Chem. *100*:485, 1933.

[6] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69, 1945.

[7] Fiske, C. H., and SubbaRow, Y.: Colorimetric determination of phosphorus. J. Biol. Chem. *66*:375-400, 1925.

[8] Barker, S. B., and Summerson, W. H.: Colorimetric determination of lactic acid in biological material. J. Biol. Chem. *138*:535, 1941.

[9] Kipnis, D. M., and Cori, C. F.: Studies on tissue permeability. III. The effect of insulin on pentose uptake by the diaphragm. J. Biol. Chem. *224*:681-93, 1957.

[10] Roe, J. H., and Rice, E. W.: A photometric method for the determination of free pentoses in animal tissues. J. Biol. Chem. *173*:507, 1948.

[11] Park, C. R., Bornstein, J., and Post, R. L.: Effect of insulin on free glucose content of rat diaphragm in vitro. Am. J. Physiol. *182*:12, 1955.

[12] Randle, P. J., and Smith, G. H.: Regulation of glucose uptake by muscle. Biochem. J. 70:490, 1958.

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## *Developmental Genetics*

A step up in the biological hierarchy is the problem of development. There is not much of a nonspeculative character that can be said of histogenesis, which rests on the still enigmatic relations of genes and cytoplasm to which we have referred. With respect to morphogenesis there is at least increasing contact between geneticists and embryologists. There was a period not long ago when experimental embryologists would trace the interactions of various factors in the development of an organ—pressures and tensions, inductions, hormones, neural stimulation, environmental conditions—and perhaps, at the end, list heredity as a sort of magic that operated through other than physiological channels. More recently, many trained embryologists have turned to genetically determined abnormalities as useful material, with illuminating results. The systematic study of the interaction effect of numerous loci on particular characters is especially promising.

The complete analysis of the development of a higher organism nevertheless remains one of the most intractable problems of science. Perhaps it is beyond human grasp. But I suspect that there will be great advances in understanding and am sure that this can come about only in the conjunction of the two disciplines.

Beyond morphogenesis is the genetics of behavior on which a beginning has been made.

From "Genetics and the Hierarchy of Biological Sciences," by Sewall Wright, in *Science,* Vol. 130, No. 3381, pp. 959-65, Oct. 16, 1959.