GLYCOLYSIS IN PHARMACOLOGY[1, 2]

by

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Classical pharmacology deals with the action of drugs on organ systems. If the question is raised as to why a certain drug acts on a particular organ system, the answer may only be obtained by searching for some system inside the cell which is sensitive to the drug in question. The most fruitful line of endeavor has been to test the affect of the drug on enzyme systems known to be involved in cellular metabolism. Many pharmacological actions of drugs can be explained in this manner. For example, the pharmacological properties of vitamins, physostigmine, BAL, and cyanide have been explained to everyone's satisfaction on an enzymatic basis. During the past war, a great deal of attention was paid to the action of antimalarial drugs, ionizing radiation and chemical warfare agents on enzymatic processes. In fact, there is a growing school in Pharmacology which has for its main purpose the localization of drug action on enzymatic processes. Some of this work has been reviewed by GREEN[1], BERNHEIM[2], CLARK[3], and MCELROY[4]. The recent book by WORK AND WORK[5] is an excellent example of the development of this field in chemotherapy.

WELCH AND BUEDING[6] have laid down very severe criteria which should be met before the action of a drug can be attributed to its effects on an enzyme system. These criteria involve concentrations, organ and tissue specificity and close parallelism between the activity of structurally related compounds. These criteria are very hard to meet in this field. It is very difficult to determine how much drug is acting on a specific organ when the drug is administered to the whole animal. When working on enzyme systems, cell interfaces are destroyed and permeability is no longer a question, which may modify drug action. Therefore, the criteria of WELCH AND BUEDING[6] should be used as an ultimate goal and not be used to delay or to give up work and thinking in this field.

It is the purpose of this article to give several examples of drug action on the glycolytic system in order to show how the discoveries of MEYERHOF are now being used in Pharmacology. MEYERHOF[7] used many pharmacological agents as chemical tools in his work on muscle metabolism. Narcotics, methylene blue, chloroform, caffeine, and moniodoacetic acid are a few of many agents employed in his work. More recently MEYERHOF and his associates have employed alloxan[8] in their study of glycolysis of brain preparations and have reported[9] the effects of potassium 1, 2-naphthoquinone-4-sulfonate on the respiration and glycolysis of *Trypanosoma equiperdum*.

[1] Read before a *Seminar at the Army Chemical Center*, March 9, 1949.

[2] In this paper, the term "glycolysis" is used in the general meaning for the break down of any carbohydrate into lactic acid by enzymatic processes.

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Any abnormal cell, invading organism or abnormal metabolic event in the body involving or using carbohydrate opens itself to this mode of attack, namely, to find a chemical substance which will block or modify its use of carbohydrate but not affect the use of carbohydrate by the normal cells of the host. In this manner the abnormal cells or invading organisms can no longer use sugar for energy purposes and thus are destroyed. Abnormal metabolism of carbohydrate may also be checked or diverted into normal pathways in a similar manner. Since the carbohydrate is generally oxidized by the invading organisms, two possibilities are available for blocking by enzymatic inhibitors; a) in the oxidative chain and b) in the glycolytic system. In the cancer field, for example, if an agent could be found which will block the use of glucose either by oxidation or by glycolysis in the rapidly growing cells, growth would cease since these cells depend mainly on the metabolism of glucose for their growth. Therefore, there should be a constant search for compounds which inhibit glycolysis or the oxidation of various sugars. Such a search may some day be rewarded with a differential inhibitor which will block sugar utilization in the cancerous cell and not in the normal cell. Such inhibitors have been found already for certain invading organisms and may well be found for the cancer cell. A review of some of the literature in this field up to 1938 has been made by GEMMILL[10].

Quinine and Atabrine: During the war, EVANS and his associates made a very intensive study of quinine and atabrine on glycolysis. This group demonstrated that the glycolysis of the malarial parasite was similar to that of the phosphorylating glycolysis of yeast and muscle[11]. Following these observations the effects of quinine and atabrine were investigated[12] on this system from malarial parasites, yeast and mammalian muscle. Atabrine inhibited hexokinase activity and the lactate dehydrogenase in the parasite preparations. Both quinine and atabrine inhibited the yeast hexokinase while quinine was inhibitory to the phosphorylase and the phosphoglucomutase from rabbit's muscle. Lactate dehydrogenase from beef heart was very susceptible to atabrine action. However, from the concentrations needed to inhibit these enzymes in the glycolytic systems, these authors concluded that the therapeutic site of inhibition is probably in the oxidative cycle unless there is a possibility of a high concentration of these drugs localizing inside the parasite cell. BOVARNICK, LINDSAY, AND HELLERMAN[13] attribute the inhibitory action of atabrine on the oxidation of glucose to an interference of phosphorylation which is essential before glucose may be oxidized by the malarial parasite.

Naphthoquinones: There has been considerable attention given to the naphthoquinones in pharmacology in recent years. In addition to the discovery that vitamin K has a naphthoquinone nucleus, these compounds have been investigated for their antimalarial[14], fungicidal[15], antitubercular[16], and antibacterial actions[17]. Some of the naphthoquinones have the power to inhibit mitosis which makes them of interest from the standpoint of tumor growth[18]. Naphthoquinones inhibit acid formation in the saliva which may aid in the prevention of tooth decay[19].

Considerable work has been done to explain the action of naphthoquinones on a possible enzymatic site. WENDEL[20] has described an inhibition of the oxygen uptake and the use of carbohydrate in red blood cells parasitized with a malarial parasite. BALL, ANFINSEN, AND COOPER[21] have made an extensive study of the inhibition of oxygen uptake and have come to the conclusion that the inhibitory site is between cytochrome c and b in the chain of respiratory enzymes. BUEDING, PETERS, AND WAITE[22]

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have shown that 2-methyl-1,4-naphthoquinone inhibits aerobic glycolysis in *Schistosoma mansoni, in vitro*. WARREN[23] has observed a similar effect in bone marrow. MEYERHOF AND RANDALL[9] have found an inhibition of respiration, glycolysis and motility of *Trypanosoma equiperdum, in vitro*, using potassium 1,2-naphthoquinone-4-sulfonate. GEMMILL[24] has studied the effects of various naphthoquinones on anerobic glycolysis of frog muscle. His results are given in Table 1.

TABLE I

NAPHTHOQUINONES WHICH INHIBITED GLYCOLYSIS IN CONCENTRATIONS OF $1 \cdot 10^{-3}$ MOLAR OR LESS

1. Sodium 1,2-naphthoquinone-4-sulfonate
2. 2-methyl-1,4-naphthoquinone
3. Sodium 2-methyl-1,4-naphthohydroquinone diphosphate
4. 2-hydroxy-3-methyl-1,4-naphthoquinone (Phthiocol)
5. 2-methyl-4-amino-1-naphthol hydrochloride
6. 2-hydroxy-1,4-naphthoquinone (Lawsone)
7. 1,4-naphthohydroquinone
8. 2-methyl-3-bromo-1,4-naphthoquinone
9. 2-chloro-3-N-thiobutyl-1,4-naphthoquinone
10. 2-methyl-3-thioethyl-1,4-naphthoquinone
11. 2-hydroxy-3-cyclohexanol-1,4-naphthoquinone

In Table I may be seen several napthoquinones which are glycolytic inhibitors. The relationship of concentration to inhibition by sodium 1,2-naphthoquinone-4-sulfonate may be seen in Fig. 1. At low concentrations there is a slight stimulation of glycolysis. As the concentrations increase there is a marked change in glycolysis with practically complete inhibition occurring with concentration of $0.4 \cdot 10^{-3}$ Molar. Some of the naphthoquinones which have vitamin K activity also are inhibitors of anerobic glycolysis: 2-methyl-1,4-napthoquinone, sodium 2-methyl-1,4-naphthoquinone diphosphate and 2-methyl-4-amino-1-naphthol hydrochloride. Another interesting fact which came out of this work was that the attachment of a halogen in the 2 or 3 position increased the inhibitory activity of these compounds.

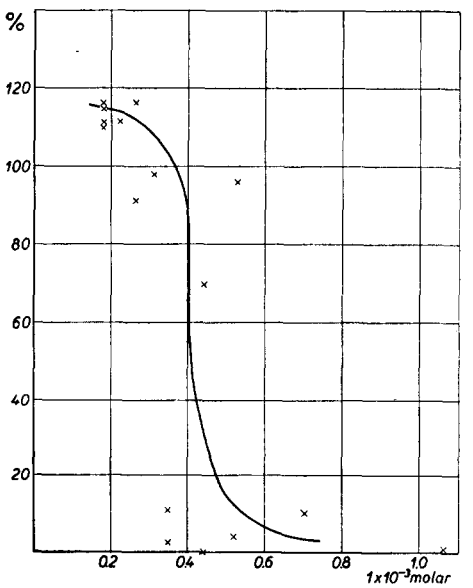

Fig. 1. The effects of increasing concentrations of sodium 1,2-naphthoquinone-4-sulfonate on glycolysis. Abscissae, $1 \cdot 10^{-3}$ Molar final concentration: ordinates, per cent of normal glycolysis.

Amidines and Related Compounds: Historically, the study of the chemotherapeutic properties of the diamidine compounds was a direct result of a search for agents which would block the use of glucose by the trypanosomes[25]. The early discovery that decamethylene diguanidine hydrochloride (Synthalin) was effective against certain trypanosomes led to a search for less toxic substances. Out of this search came many guanidines, isothioureas, amidines[26] and numerous aromatic diamidines, among them being stilbamidine and pentamidine. It was soon

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shown that doses of the diamidines which were active against trypanosomes did not produce a fall in blood sugar of the host. Therefore, attention was given to the sugar metabolism and oxygen utilization of these organisms. LOURIE AND YORKE[27] have stated that the diamidines may block the aerobic glucose metabolism in the diamidine-sensitive species. The diamidine-insensitive species would be capable of obtaining their energy from the anerobic glycolysis in the presence of the drug.

Some attention has been paid to the possible enzymatic site of the action of these compounds. BLASCHKO AND DUTHIE[28] have found an inhibitory action of the various amidine derivatives on the amine oxidase activity of the rabbits' liver. BERNHEIM[29] has shown that the oxidation of proline and alanine by *E. coli* is inhibited by propamidine. However, the oxidation of glucose, pyruvate and succinate is not affected by this drug. DICKENS[30] has demonstrated that guanidine carbonate increases the aerobic glycolysis of the rat brain cortex. These facts led to a study of the effects of diamidines and related compounds on anerobic glycolysis of glycogen to lactate in muscle extract (GEMMILL[31]). The various compounds in this series which inhibited glycolysis are given in Table II. In the same paper is given a list of styryl and cyanine compounds which are active inhibitors.

TABLE II

AMIDINES AND RELATED COMPOUNDS WHICH INHIBITED GLYCOLYSIS IN CONCENTRATIONS OF $1 \cdot 10^{-3}$ MOLAR OR LESS

Diamidines:	Diguanidines:
C_{12}·2 HCl	Diguanidine HCl
C_{13}·2HCl	C_{12} HCl
Monoguanidines:	Diisothioureas:
Guanidine HCl	C_{10} HBr
Methylguanidine sulfate	C_{12} HBr
Arginine HCl	Stilbamidine
C_8 HCl	Pentamidine
C_{13} HCl	Chlorguanidine

Alloxan: Since the discovery that alloxan may produce diabetes by destroying the cells in the islets of LANGERHANS, there has been a renewed interest in the effect of alloxan on enzyme systems. PURR[32] has demonstrated that alloxan has the ability to inhibit papain and cathepsin and HOPKINS, MORGAN, AND LUTWAK-MANN[33] have shown the same effect on the succinic dehydrogenase. Alloxan may act as a hydrogen acceptor in enzyme solutions[34, 35]. GEMMILL[36] has demonstrated that alloxan may inhibit glycolysis. The degree of inhibition was proportional to the concentration of alloxan and the inhibition was partially reversed by cysteine. Therefore alloxan may be added to the group of oxidizing agents which can reversibly inactivate glycolysis. It would be of interest to show that the cells in the islets of LANGERHANS have a glycolytic system which was very sensitive to this reagent.

Caffeine: Considerable work has been done on the effect of caffeine on glycolysis in the intact muscle. MEYERHOF[37] demonstrated that caffeine increased lactate formation. MATSUOKA[38] continued and reported in detail this demonstration. DAVID[39] has shown a large increase in lactate formation in caffeine contracture. GEMMILL[40], in cell free extracts, was able to demonstrate that caffeine and some theobromine derivatives caused an increase in the rate of glycolysis which was followed by an inhibition.

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Mercury Compounds: GEMMILL AND HELLERMAN[41] studied the effects of small concentrations of phenylmercuric hydroxide, p-chloromercuric benzoic acid and mercuric chloride on glycolysis in muscle extracts. These substances have the power to inhibit glycolysis and the inhibition is abolished by the addition of cysteine.

Iodine: In the same paper in which the action of the mercury compounds on glycolysis was described, GEMMILL AND HELLERMAN[41] also demonstrated that small amounts of iodine inhibited glycolysis. This effect was reversed by the addition of cysteine. LIPMANN[42] had previously shown that iodine was an active inhibitor of glycolysis. RAPKINE[43] traced the action of oxidizing agents to the oxidoreduction between phosphoglyceraldehyde and pyruvic acid. LIPMANN[44] has pointed out that there are five enzymes in the glycolytic system which may undergo oxidative inactivation and reactivation with glutathione.

Anesthetics: WATTS[45], working in this laboratory, has shown that methadon and nupercaine have the property of maintenance of glycolysis over and above the normal velocity of this process in an activated homogenate of rat brain. During the first ten minutes, there is no difference in the rate of glycolysis. However, after the first ten minutes, the normal rate tends to decrease, while the mixture containing either of these two drugs maintains the same rate of the original ten minute period. Using radioactives phosphorous in the form of the phosphate ion, PERTZOFF AND GEMMILL[46] have shown that sodium barbital and ether have a retarding effect on the transfer of phosphate from plasma into the red blood cell.

SUMMARY

Several examples of the action of chemical compounds of therapeutic interest on glycolysis have been given in this short review. In most of these cases, the methods and results of Professor MEYERHOF have been used as a background in this work. Many developments are possible from this type of work, especially in the explanation of drug action and the control of disease through this knowledge. Therefore, pharmacology owes much to the pioneer investigations of Professor MEYERHOF.

RÉSUMÉ

Dans cette brève revue nous avons donné plusieurs exemples de l'action sur la glycolyse de certains composés chimiques d'intérêt pharmaceutique. Dans la plupart des cas les méthodes et les résultats du Professeur MEYERHOF ont formé le point de départ de ce travail. Ce genre de travail peut donner lieu à des développements nombreux, surtout pour expliquer l'action des drogues et, par ce fait, pour enrayer la maladie. C'est pourquoi la pharmacologie doit beaucoup aux investigations de pionnier du Professeur MEYERHOF.

ZUSAMMENFASSUNG

In dieser kurzen Übersicht wurden einige Beispiele für die Wirkung chemischer Verbindungen von therapeutischem Interesse auf die Glykolyse gegeben. In den meisten Fällen bildeten die Methoden und die Ergebnisse von Professor MEYERHOF den Hintergrund dieser Arbeit. Vielerlei Entwicklungen dieser Arbeit sind möglich, insbesondere zur Erklärung der Wirkung der Arzneimittel und dadurch zur Eindämmung der Krankheiten. Deshalb hat die Pharmakologie den Pioniersuntersuchungen von Professor MEYERHOF viel zu verdanken.

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