cretion time is not modified by CYN in doses equivalent to the doses which may be employed in clinical therapy (BERTOLANI *et al.*[7]), in contradiction to other choleretics, which may impair the excretory function of the liver (DELCOURT[8,9]).

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Department of Pharmacology, University of Naples (Italy), December 1, 1958.

Riassunto

L'attività coleretica, nel ratto, dell'ac. 1,4 dicaffeilchinico (Cinarina) risulta per dosi di 15–30 mg/kg per intensità e durata sovrapponibile a quella del Na-deidrocolato in dosi equimolecolari, per dosi più elevate (75 a 100 mg/kg) più prolungata. La funzione escretrice del fegato non è sfavorevolmente influenzata dalla Cinarina.

[7] F. BERTOLANI, M. DARDARI, and L. MASSA, *Significato e valore pratico della utilizzazione di un farmaco ad azione colocrina, l'ac. 1,4-dicaffeilchinico, nella indagine colangiocolecistografica con mezzo di contrasto endovenoso (ricerca sperimentale e clinica)* (Casa Ed. Ambrosiana, Milano 1957).

[8] A. DELCOURT and A. DOMB, J. Physiol. *48*, 494 (1956).

[9] A. DELCOURT, Ann. Soc. R. Sci. méd. natur. Bruxelles *9*, 281 (1956).

Phenothiazine Derivatives as Inhibitors of the Glucose Oxidative Pathway in Human Erythrocytes

It has been previously noted that human erythrocytes in the presence of methylene blue oxidize glucose by way of phosphogluconate oxidation[1]. The oxidation of glucose is increased 20 to 50 times over the low level established without the dye. The enzymes necessary for the oxidation of glucose to ribose-5-phosphate are present in the erythrocyte[2]. The oxidative steps of the pentose phosphate pathway, because of the ability to produce TPNH[3], have an important function in cell growth and synthesis[4].

In view of the above, and because of an interest in the effects of tranquilizing agents on enzyme systems, the behaviour of three phenothiazine derivatives on phosphogluconate oxidation in erythrocytes was investigated.

Human blood, collected in acid citric-dextrose (Formula B, National Institutes of Health) was obtained from the local Red Cross. The cells were prepared according to HUENNEKENS *et al.*[5]. Since there is a loss in the ability of the cells to oxidize glucose when they are stored several weeks, without any decrease in hexokinase activity[6], adenosine (2100 μM/100 ml of cells) was added prior to storage of the erythrocytes at 4° C. Adenosine triphosphate declines during storage and is regenerated upon the addition of adenosine[5].

[1] O. WARBURG, F. KUBOWITZ, and W. CHRISTIAN, Biochem. Z. *227*, 245 (1930).

[2] F. LIPMANN, Nature *138*, 588 (1936).

[3] TPN – Triphosphopyridine nucleotide; TPNH – reduced TPN.

[4] B. L. HORECKER and H. H. HIATT, New England J. Med. *258*, 225 (1958).

[5] F. M. HUENNEKENS, L. LIEU, H. P. A. MYERS, and B. W. GABRIO, J. biol. Chem. *227*, 253 (1957).

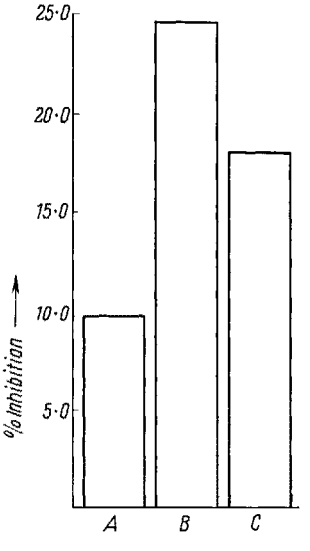
[6] B. W. GABRIO, C. A. FINCH, and F. M. HUENNEKENS, Blood *11*, 103 (1956).

Glucose oxidation in whole cells was measured by the standard Warburg technique. The system as employed by HUENNEKENS[5] was used. The phenothiazines were added at the expense of the Krebs-Ringer phosphate solution. Both cells and drugs were added initially to the main compartment of the Warburg flask and were incubated together 15 min before starting the experiment.

Effect of Inhibitors on Enzyme Activity

Drug	Conc. $\times 10^{-4} M$	% Inhibition
Promazine	5·0	16·0
	2·5	10·0
	1·25	1·4
SKF 5883	5·0	48·8
	2·5	25·5
	1·25	13·8
Adazine	5·0	51·3
	2·5	21·8
	1·25	12·3

The Table indicates the inhibiting effects of the three phenothiazines studied. At equivalent molar concentrations, adazine [10-(3-dimethylaminopropyl)-2-trifluoromethyl phenothiazine hydrochloride] was the most effective in preventing oxygen consumption by the cells followed by SKF 5883 (N,N-dimethyl-10-[3-(1-methyl-4-piperazinyl)-propyl]-2-phenothiazine sulfonamide dimethanesulfonate) and promazine [10-(3-dimethylaminopropyl)-phenothiazine hydrochloride]. It was also observed that the inhibiting effect of drugs increased with increasing concentration of the drug. These results resemble those found in other systems[7].



Effect of Drug Combination on Enzyme Activity
A = Promazine at $2{\cdot}5 \times 10^{-4} M$;
B = SKF 5883 at $2{\cdot}5 \times 10^{-4} M$;
C = Combination of the two, each at $2{\cdot}5 \times 10^{-4} M$

Additional observations were made in which two of the drugs were present in the same reaction mixture at $2{\cdot}5 \times 10^{-4} M$. As indicated in the Figure, these drugs do

[7] E. W. HELPER, M. J. CARVER, H. P. JACOBI, and J. A. SMITH, Arch. Biochem. Biophys. *76*, 354 (1958).

not have an additive effect but inhibit glucose oxidation to a value intermediate between those obtained when the drugs are used alone at these concentrations. Similar results were obtained with other combinations.

Previous work has indicated that some of these compounds inhibit cytochrome oxidase from rat liver and brain [7]. Reduced triphosphopyridine nucleotide is oxidized slowly by this system. However, TPNH oxidase is present in the erythrocyte and may possibly be the specific enzyme inhibited by these compounds. These experiments were not designed to establish this.

The molar concentrations required in this study were in excess of the pharmacological dose. However, it is possible that the inhibition noted may be the basis for other clinical effects of the drugs. Further work is in progress on the use of human red blood cells in studying enzyme inhibition by phenothiazine derivatives.

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M. J. Carver and Nadine Roesky

Neurochemical Laboratory, Nebraska Psychiatric Institute, College of Medicine, Omaha (Nebraska), December 1, 1958.

Zusammenfassung

Phenothiazinabkömmlinge unterscheiden sich in ihrer Wirksamkeit als Hemmstoffe im oxydativen Glukosestoffwechsel in menschlichen Erythrozyten. Kombiniert man verschiedene Phenothiazinderivate, so erhält man einen mittleren Hemmungsgrad, der zwischen jenen liegt, die man bei Anwendung der einzelnen Wirkstoffe für sich allein erhalten würde. Somit erscheint es nicht wahrscheinlich, dass Hemmung des oxydativen Glukosestoffwechsels die prinzipielle Wirkungsweise der Phenothiazinabkömmlinge darstellt.

[8] SKF-5883 was kindly supplied by Smith, Kline and French Laboratories, Philadelphia; Promazine by Wyeth Laboratories, Philadelphia; and Adazine by the Upjohn Company, Kalamazoo.

The Extractibility of Calf Thymus Histone Fractions

It is well known that the calf thymus histone is composed of at least two main fractions. The arginine-rich fraction contains approximatively five times more tyrosine than the lysine-rich one [1–4]. The difference in the solubility of the sulphates of these two main fractions of calf thymus histone was used by some authors for its fractionation[4,5].

The nuclear material was prepared by modified extraction of the cytoplasm with 0·14 *M* NaCl according to Butler *et al.*[6]. Histone was extracted with diluted hydrochloric acid in two ways: in the first, the concentration of the acid, when mixed with nuclear material, amounted to 0·1 *N*; in the second to 0·2 *N*. Apart from this method, histone was isolated by extraction of the bulk of proteins

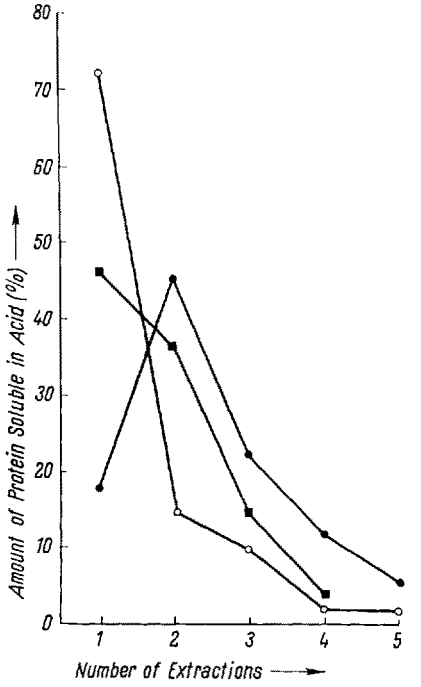

Fig. 1.—The amount of acid-soluble protein (histone) in subsequent extractions of nuclear material, prepared by using 0·14 *M* NaCl extracted with 0·1 *N* HCl (●—●—●) and 0·2 *N* HCl (o—o—o) and of the material prepared according to Sevag *et al.*[7] and extracted with 0·1 *N* HCl (■ ■—■)

remaining after isolation of desoxyribonucleic acid (DNA), according to Sevag *et al.*[7]. This protein-containing material was also extracted with 0·1 *N* hydrochloric acid. For the complete removal of all the protein soluble in diluted hydrochloric acid, five extractions are necessary (Fig. 1).

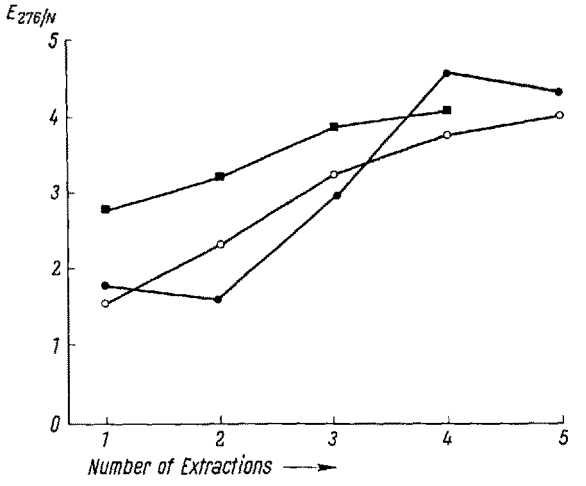

Fig. 2.—The E_{276}/N ratios of subsequent extractions of calf thymus histone. Histone extracted with 0·1 *N* HCl from the nuclear material prepared by using 0·14 *M* NaCl (●—●—●), histone prepared from the same material extracted with 0·2 *N* HCl (o—o—o) and histone extracted with 0·1 *N* HCl from the proteins resulting from the Sevag process (■—■ – ■)

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