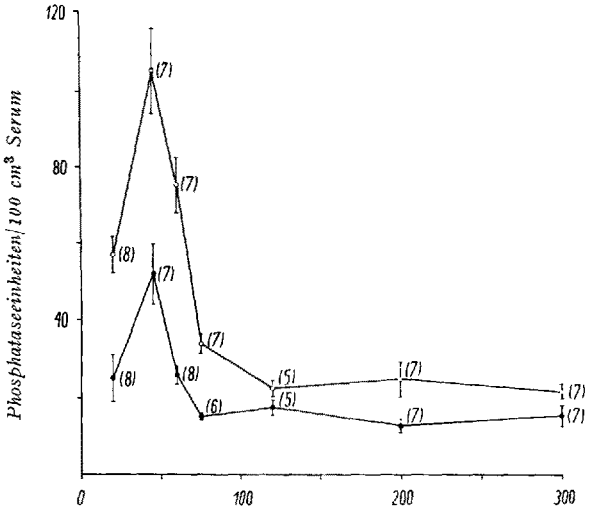
Menschen beobachteten Aktivitätsveränderungen sollten bei der Ratte, einem Tier mit lebenslänglichem Wachstum, untersucht werden. So wurden 1. die Serumphosphataseaktivitäten bei 52 männlichen Albinoratten im Alter von 20, 45, 60, 75, 120, 200 und 300 Tagen, 2. die Körpergewichtszunahme dieser Tiere und 3. der Einfluss der Kastration auf die Serumphosphatasen geprüft. Bei den etwa 18 h fastenden Tieren sind die Phosphatasen nach der HUGGINS-TALALAYschen Methode[4] wie bereits beschrieben bestimmt worden[5]. Am gleichen Tag wurden jeweils Ratten verschiedener Altersgruppen in den Versuch genommen. Um jahreszeitliche Einflüsse auszuschalten, wurden alle Beobachtungen im Monat Mai durchgeführt.




Die Aktivität der Serumphosphatasen männlicher Ratten in Abhängigkeit vom Alter. o——o = alkalische, •——• = saure Serumphosphatase (Mittelwerte). I = Standardfehler des Mittelwertes. In Klammern Anzahl der Tiere

*Phosphataseaktivität und Lebensalter.* Die Aktivitäten der alkalischen und sauren Phosphatase (Abb.) wachsen im allgemeinen parallel bis zu einem Maximum im Lebensalter von 45 Tagen an, um danach wieder abzunehmen. Die statitische Auswertung wurde durch den Vergleich des Durchschnittswertes der Altersgruppe von 45 Tagen (höchste Phosphatasenaktivität) mit den durchschnittlichen Werten von allen übrigen Altersgruppen unter Anwendung des «*t*»-Testes durchgeführt. Diejenigen Einzelbeobachtungen (weniger als 4%), bei denen die quadratische Abweichung erheblich mehr als die Hälfte der Summe aller Quadratabweichungen betrug, wurden nach SEALEY und SONDERN[6] als aberrant angesehen. Die Abbildung enthält diese Werte nicht. Die Aktivitätszunahme der alkalischen Phosphatase vom 20. bis zum 45. Tag ist signifikant ($p < 0{,}01$). Vom 45. bis zum 60. Tag nimmt sie wahrscheinlich signifikant ($p < 0{,}05$) und bis zum 75. Tag stark signifikant ($p < 0{,}001$) ab. Die Aktivitätswerte stabilisieren sich nach dem 120. Tag und zeigen hierauf keine signifikanten Veränderungen mehr bis zum 300. Alterstag. Grundsätzlich gilt dasselbe für die saure Serumphosphatase. Sie nimmt vom 20. Tag bis zum 45. Tag auch signifikant zu ($p < 0{,}02$), sinkt dann bis zum 60. Tag hoch signifikant ab ($p < 0{,}001$), um nach Ablauf einer 75tägigen Lebensdauer praktisch unverändert zu bleiben. Zur Zeit des Aktivitätsmaximums ist die Variabilität für beide Phosphatasen am grössten; die Ursache dafür könnte vielleicht darin liegen, dass die verschiedenen Tiere nicht in genau derselben Zeit die höchste Phosphataseaktivität erreichen.

*Phosphataseaktivität und Zuwachsrate.* Die Zuwachsrate unserer Versuchstiere zeigt gewöhnlich im Alter von 75 Tagen maximale Werte und stimmt nicht mit der Zeit der höchsten Phosphataseaktivität überein. Diesbezüglich sind weitere Untersuchungen notwendig, da die Körpergewichtzunahme nicht als zuverlässiges Kriterium für das Wachstum des Skelettes dienen kann.

*Phosphataseaktivitäten und Keimdrüsen.* Nachdem die Abnahme der Phosphataseaktivitäten in die Zeit der sexuellen Reifung der Ratte fällt, wurden die Serumphosphatasen bei kastrierten und normalen männlichen, etwa 100 Tage alten Ratten – also zur Zeit, in welcher bei unseren Tieren die Aktivitäten der sauren Serumphosphatase schon stabilisiert sind – bestimmt. Sechs Normalratten zeigten eine saure Phosphataseaktivität von $25{,}5 \pm 3{,}7$ Einheiten (Standardfehler des Mittelwertes). Bei 8 Ratten, die 40 Tage vorher kastriert wurden, stieg die saure Phosphataseaktivität auf $46{,}0 \pm 2{,}7$ Einheiten (hoch signifikant; $p < 0{,}001$) und erreichte damit fast die maximalen Werte unserer Tiere. Interessanterweise wurde die alkalische Serumphosphatase durch die Kastrierung nicht beeinflusst. Zur weiteren Klärung der Frage sollte die Kastrierung in verschiedenen Lebensaltern unternommen werden.

Für die technische Mithilfe sind wir Herrn GLUŠČEVIĆ besonders verpflichtet.

L. RABADJIJA, M. VRANIĆ,
N. ALLEGRETTI und N. POKRAJAC

*Physiologisches Institut, Medizinische Fakultät der Universität Zagreb (Jugoslawien), 21. Oktober 1958.*

*Summary*

The serum alkaline and acid phosphatases were examined in male rats between the ages of 20 and 300 days, using the method of HUGGINS and TALALAY[4]. The activities of acid and alkaline phosphatases increase significantly to the age of 45 days, followed by a highly significant decrease up to 120th day for alkaline and to 75th day for acid phosphatase. After castration, highly significant increases in the acid, but no changes in the alkaline serum phosphatase were observed.

[4] CH. HUGGINS und P. TALALAY, J. biol. Chem. *159*, 399 (1945). – K. LINHARDT und K. WALTER, Hoppe Seyler's Z. *289*, 245 (1952).

[5] M. VRANIĆ, N. ALLEGRETTI und L. RABADJIJA, Hoppe Seyler's Z. *314*, 1 (1959).

[6] J. L. SAELEY und C. W. SONDERN, Endocrinology *26*, 813 (1940).

## Comparison of Choleretic Effects of CYN and Na-Dehydrocholate

1,4 dicaffeylquinic acid (Cynarine or CYN), originally extracted from the large basal leaves of artichoke and later also prepared by synthesis[1], was found to be the active principle of artichoke (PREZIOSI *et al.*[2–4]).

[1] L. PANIZZI, M. L. SCARPATI, and R. SCARPATI, Gazz. chim. ital. *84*, 806 (1954).

[2] P. PREZIOSI and B. LOSCALZO, Fistoterapia *27*, 666, 690 (1956).

[3] P. PREZIOSI and B. LOSCALZO, Arch. Ital. Sci. Farmacol. *7*, 244 (1957).

[4] P. PREZIOSI and B. LOSCALZO, Arch. int. Pharmacodyn. *117*, 63 (1958).

*Table I*

| Animal No. | Treatment | | | Amount of bile in ml ± s.e. secreted during | | | | | Percentage variations compared with basal secretion | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Substance | mg/kg | ml/kg | 1st h | 2nd h | 3rd h | 4th h | 5th h | 2nd h | 3rd h | 4th h | 5th h |
| 12 | — | — | — | 0·35 ± 0·010 | 0·31 ± 0·027 | 0·30 ± 0·021 | 0·32 ± 0·030 | 0·29 ± 0·032 | – 11·5 | – 14,3 | – 8·6 | – 17·2 |
| 8 | Ph. B. *M*/5 | — | 5 % soln. | 0·42 ± 0·038 | 0·38 ± 0·037 | 0·35 ± 0·061 | 0·32 ± 0·049 | 0·29 + 0·033 | – 9·6 | – 16·7 | – 23·9 | – 31 |
| 5 | Na-dehydrocholate | 5·53* | 0·11 | 0·48 ± 0·049 | 0·51 ± 0·055 | 0·54 ± 0·051 | 0·48 ± 0·04 | 0·42 ± 0·023 | + 6·2 | + 12·5 | 0 | – 12·5 |
| 8 | Na-dehydrocholate | 11·85 | 0·22 | 0·28 ± 0·029 | 0·35 ± 0·022 | 0·39 ± 0·032 | 0·28 ± 0·014 | 0·25 ± 0·010 | + 25 | + 39·2 | 0 | – 10·8 |
| 9 | Na-dehydrocholate | 23·7 | 0·57 | 0·38 ± 0·028 | 0·52 ± 0·040 | 0·39 ± 0·026 | 0·34 ± 0·019 | 0·33 ± 0·018 | + 36·8 | + 2·6 | – 10·6 | – 13·2 |
| 9 | Na-dehydrocholate | 39·5 | 0·79 | 0·42 ± 0·040 | 0·72 ± 0·044 | 0·38 ± 0·037 | 0·31 ± 0·072 | 0·24 ± 0·045 | + 71·4 | – 9·6 | – 26·2 | – 42·9 |
| 8 | Na-dehydrocholate | 59·25 | 1·19 | 0·29 ± 0·006 | 0·64 ± 0·042 | 0·28 ± 0·035 | 0·28 ± 0·049 | 0·20 ± 0·040 | + 120·6 | – 3·5 | – 3·5 | – 31·1 |
| 8 | Na-dehydrocholate | 79 | 1·58 | 0·34 ± 0·019 | 0·78 ± 0·011 | 0·41 ± 0·017 | 0·28 ± 0·017 | 0·25 ± 0·026 | + 129·4 | + 20·5 | – 17·7 | – 26·5 |
| 6 | CYN | 7 | 0·14 | 0·47 ± 0·05 | 0·50 ± 0·06 | 0·46 ± 0·07 | 0·43 ± 0·036 | 0·45 ± 0·023 | + 6·3 | – 2·2 | – 8·6 | – 4·3 |
| 5 | CYN | 15 | 0·30 | 0·46 ± 0·07 | 0·57 ± 0·09 | 0·53 ± 0·05 | 0·42 ± 0·02 | 0·42 ± 0·04 | + 23·9 | + 15·2 | – 8·7 | – 8·7 |
| 6 | CYN | 30 | 0·60 | 0·31 ± 0·11 | 0·44 ± 0·06 | 0·35 ± 0·05 | 0·29 ± 0·036 | 0·31 ± 0·11 | + 41·9 | + 12·9 | – 6·5 | 0 |
| 5 | CYN | 50 | 1 | 0·42 ± 0·02 | 0·63 ± 0·05 | 0·51 ± 0·03 | 0·39 ± 0·043 | 0·34 ± 0·012 | + 50 | + 21·4 | – 7·2 | – 19·1 |
| 6 | CYN | 75 | 1·5 | 0·50 ± 0·02 | 0·96 ± 0·1 | 0·65 ± 0·06 | 0·56 ± 0·061 | 0·50 ± 0·002 | + 92 | + 30 | + 12 | 0 |
| 5 | CYN | 100 | 2 | 0·40 ± 0·01 | 0·78 ± 0·04 | 0·60 ± 0·05 | 0·49 + 0·037 | 0·44 ± 0·06 | + 95 | + 50 | + 22·5 | + 10 |

* 5·53 – 11·85 – 23·7 – 39·5 – 59·25 – 79: equimolecular doses to 7 – 15 – 30 – 50 – 75 – 100 mg/kg CYN respectively. s. e. = standard error

CYN possesses choleretic and diuretic properties and has also some interesting effects on cholesterol metabolism.

This paper deals with the results of a series of experiments in which some aspects of the choleretic action of CYN, e.g. dose-effect relation, duration of action, influence on excretion time of bromesulfonephtaleine (B. S. P.) were compared with the same effects exerted by Na-dehydrocholate (NaDC).

*Material and methods.* Albino rats of the Morini strain weighing 160–220 g were used. Animals of both sexes, but not pregnant females, were employed. CYN[5] and reagent grade NaDC have been used. Both substances were dissolved in phosphate buffer (Ph. B.) *M*/5 ($Na_2HPO_4$ 7·1% solution). With this solvent, a 1–2% solution of CYN may be obtained by heating on a boiling water-bath. These solutions precipitate on cooling. Their pH is about 7 (6·9–7·2). The strength of the CYN and NaDC solutions was calculated in order to inject the required amount of drug in a total volume of 5 ml liquid per kg. Groups of 10–30 rats treated only with Ph. B. (5 ml/Kg), or kept under observation without any treatment, served as controls.

In all our experiments, we used the technique of temporary biliary fistula, described by PREZIOSI and LOSCALZO[2]. In this series of experiments, however, the abdominal wall was sutured after the introduction of the plastic tube into the common biliary duct. Drugs were administered by intravenous injection. The bile flow has been controlled and measured 1 h before and 4 h after the administration of CYN, NaDC or Ph. B. In the controls without any treatment, the flow was recorded for 5 h.

[5] CYN was kindly supplied by Farmitalia.

*Results.*

1. *Dose-effect relation.* As shown in Table I, CYN may cause a 90% increase of basal biliary flow. For doses up to 50 mg/kg of CYN, as well as for equimolecular doses of

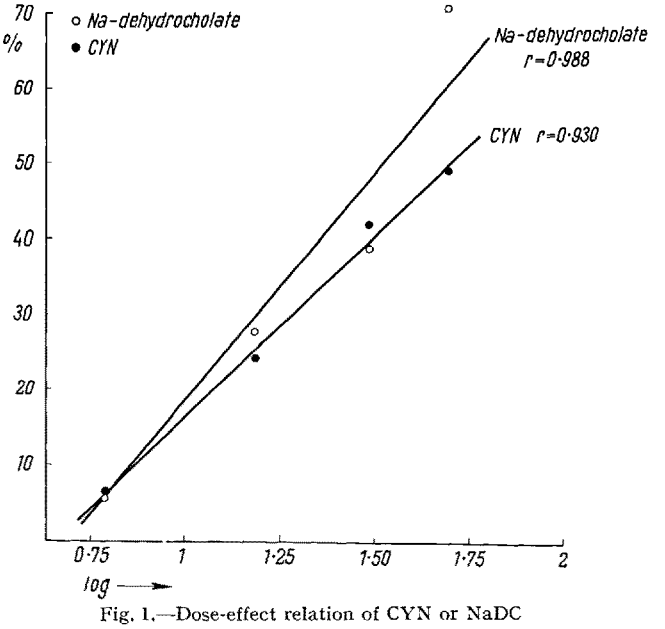



Fig. 1.—Dose-effect relation of CYN or NaDC

The average percentage variations of the biliary flow during the first hour after injection of the drugs has been plotted against the log of the doses. The '*r*' was calculated according to GÜNTHER[6]

[6] In G. POUMEAU-DELILLE, *Techniques biologiques en endocrinologie expérimentale chez le rat* (Masson, Paris 1953), p. 161–183.

NaDC, a close dose-effect relation could be observed during the first hour after the injection (Fig. 1).

2. *Duration of action.* The choleretic effect of *lower* doses (7–15 mg/kg) of CYN during the first hour is nearly equivalent to that of equimolecular doses (5·53–11·85 mg/kg) of NaDC, but during the second hour its activity is inferior to that of NaDC. *Medium* doses (30 mg/kg) of CYN and NaDC display about the same choleretic activity during the first and second hours after the injection.

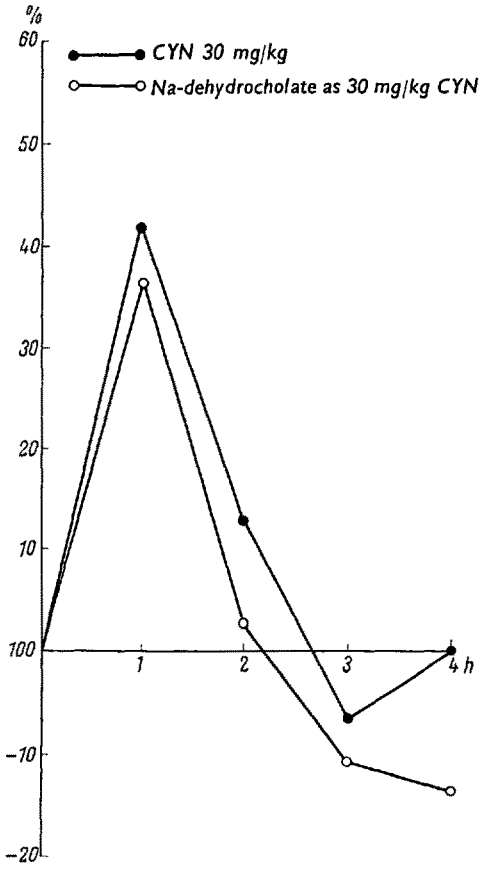



Fig. 2.—Choleresis following different doses of CYN or NaDC
The average percentage variations of the biliary flow with respect to the basal values (basal hour) have been plotted against time (after injection of drugs) during which the observation of biliary flow was performed

During the following hours, a reduction of bile flow may be observed in the animals treated with NaDC, which fails in CYN-treated rats (Table I and Fig. 2). For *higher* doses (50–75 mg/kg), the choleretic activity of CYN during the first hour is slightly inferior to that of equimolecular doses (39·5–59·25 mg/kg) of NaDC, while during the following hours the effect of CYN is more pronounced. During these hours a rather severe reduction of biliary flow below basal values may be observed in NaDC treated rats, but not in CYN-injected animals. The choleretic action of CYN in doses of 100 mg/kg lasts up to 4 h, while the choleretic effect of equimolecular doses of NaDC has a duration of about 2 h (Table I and Fig. 3).

3. *B.S.P. excretion time.* In these experiments B.S.P. (5 mg/kg, 2·5% solution) was injected intravenously 30 min after the injection of CYN or NaDC. The B.S.P. excretion time is not modified or only slightly enhanced by CYN (17–30 mg/kg), as shown in Table II. Equimolecular doses of NaDC do not influence the B.S.P. excretion time.

*Table II*

| Treatment | | BSP average excretion time min/s |
|---|---|---|
| Substance | mg/kg | |
| — | — | 4 min 45 s |
| Ph. B. *M*/5 . . . | 5 ml/kg | 4 min 30 s |
| NaDC . . . . . | 11·85 mg/kg* | 4 min 51 s |
| NaDC . . . . . | 23·7 mg/kg** | 4 min 34 s |
| CYN . . . . . | 15 mg/kg | 3 min 45 s |
| CYN . . . . . | 30 mg/kg | 4 min 12 s |

* Equimolecular to 15 mg/kg CYN.
** Equimolecular to 30 mg/kg CYN.

*Conclusion and summary.* CYN was found to have marked choleretic properties. A close dose-effect relation could be observed during the first hour after injection of the drug up to doses of 50 mg/kg. For the medium doses

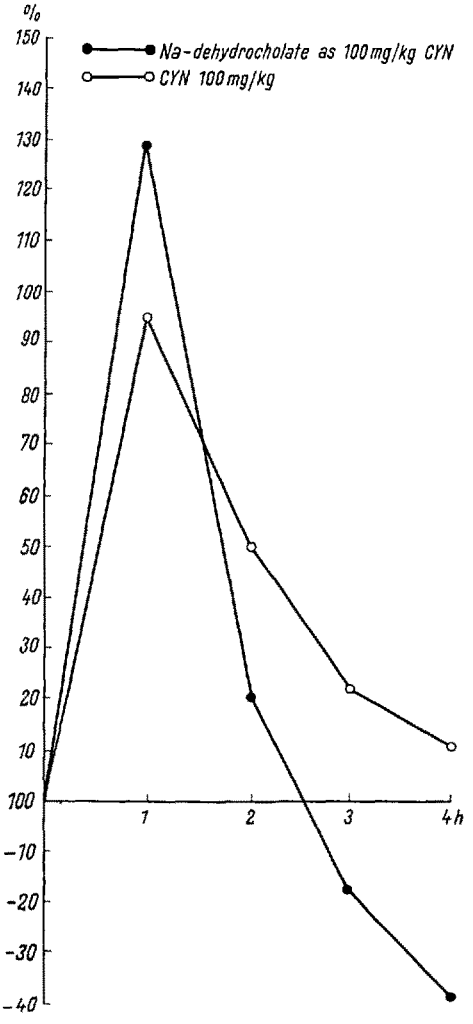



Fig. 3.—Choleresis following different doses of CYN or NaDC
The average percentage variations of the biliary flow with respect to the basal values (basal hour) have been plotted against time (after injection of drugs) during which the observation of biliary flow was performed

the choleretic effect is nearly equivalent to that of NaDC in equivalent doses, while the effect of higher doses was of longer duration, lasting up to 3–4 h. The B. S. P. ex-

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]).

P. PREZIOSI, B. LOSCALZO, and E. MARMO

*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 $\mu M$/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).

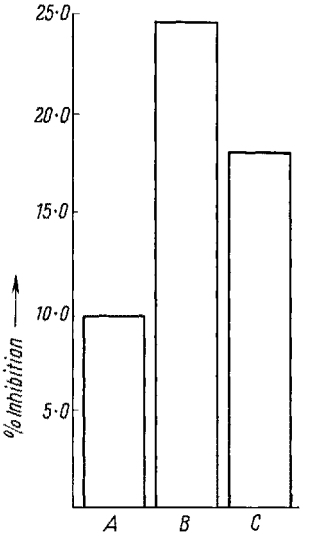
[6] B. W. GABRIO, C. A. FINCH, and F. M. HUENNEKENS, Blood *11*, 103 (1956).

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).