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ISOTHIOCYANATES. A NEW CLASS OF UNCOUPLERS

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SUMMARY

This paper describes the uncoupling effect of three isothiocyanates: *p*-bromophenylisothiocyanate, 4,4′-diisothiocyanatebiphenyl and β-naphtylmethylisothiocyanate on the respiration of Ehrlich-Lettré cells and isolated mitochondria. The isothiocyanates are similar to other uncouplers (such as 2,4-dinitrophenol and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone) in that they : 1. stimulate respiration of state 4 mitochondria; 2. stimulate mitochondrial ATPase activity; 3. release the inhibition of mitochondrial respiration by oligomycin and 4. inhibit both mitochondrial respiration and mitochondrial ATPase activity at higher molar concentrations. The uncoupling activity of these isothiocyanates correlates well with their biological activity. Maximal activation of a latent mitochondrial ATPase activity of rat liver mitochondria in the presence of *p*-bromophenylisothiocyanate was found at a concentration of 15 μM. The investigated isothiocyanates differ significantly in their solubility in organic solvents and their chemical reactivity. We assume that the greater the partition coefficient in a series of isothiocyanates grouped according to the increasing value of log *P* (partition coefficient for the system octanol/water, 25 °C), the greater will be their uncoupling activity, but only up to a certain degree. Any further increase of log *P* will be marked by a decrease of this activity.

INTRODUCTION

Natural and synthetic isothiocyanates represent a large group of sulphur-containing compounds possessing remarkable biological effects. During the last 10–15 years, more than 400 isothiocyanates have been synthesized and their physico-chemical properties have been studied [1, 2]. The biological effectiveness of natural and synthetic isothiocyanates, their mode of action and also the question of relations between

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Abbreviations: BrPh-NCS, *p*-bromophenylisothiocyanate; $(NCS)_2$-Ph_2, 4,4′-diisothiocyanatebiphenyl; Nap-CH_2NCS, β-naphtylmethylisothiocyanate; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Me_2SO, dimethyl sulphoxide; MOPS, morpholinopropane sulphonate.

the chemical structure and biological activity, have been systematically investigated by Drobnica et al. [3–11]. The structure-activity relationship of isothiocyanates have been studied in a large group of *meta-* and *para-* substituted phenylisothiocyanates [12, 13], derivatives of acridine [14] and benzylisothiocyanates [15]. A linear relationship between antibacterial activity of benzylisothiocyanate derivatives and solubility in water has been observed [16]. Reactions of isothiocyanates with amino and sulfhydryl groups of proteins and also with low molecular compounds have been studied [17, 18].

Isothiocyanates affect the processes of respiration and glycolysis. The inhibition of glycolysis has been elucidated as a consequence of inactivation of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) and hexokinase (EC 2.7.1.1). Inhibition is the result of the reaction of isothiocyanates with functional -SH groups of these enzymes. The inactivation of some dehydrogenases that participate in Krebs cycle was elucidated in a similar way [19–22]. Up to now, however, most of the experiments were performed on a cellular level and little is known about the mode of action of isothiocyanates on respiratory processes. Inhibition by aromatic isothiocyanates of the oxidations of exogeneous NADH by mitochondria from Ehrlich ascites tumor cells has been briefly reported [23].

This paper describes the uncoupling effect of three synthetic isothiocyanates : *p*-bromophenylisothiocyanate, 4,4′-diisothiocyanatebiphenyl and *β*-naphtylmethylisothiocyanate on the respiration of tumor cells and isolated mitochondria.

MATERIALS AND METHODS

Ehrlich-Lettré hyperdiploid ascites tumor cells were harvested 6–8 days after inoculation in ICR albino mice (0.2 ml ascites fluid), washed in a saline phosphate medium and suspended in the same medium as described in earlier papers (24, 25). Intact pigeon heart and rat liver mitochondria were isolated according to the method of Chance and Hagihara [26]. The protein concentration was determined by the biuret method [27]. Oxygen uptake was measured with a Clark-type oxygen electrode as described previously [25]. All mitochondrial preparations were checked for structural integrity using the criterion of respiratory control [28]. Latent mitochondrial ATPase activity was measured by a pH electrode in 0.12 M KCl and 0.02 M Tris · Cl medium [29]. The ATPase hydrolysis rate was calculated assuming 0.8 H^+ was released per ATP molecule hydrolyzed [30].

The isothiocyanates were obtained from Dr Drobnica, Department of Microbiology and Biochemistry, Slovak Polyt. University, Bratislava, Czechoslovakia and were dissolved in dimethyl sulphoxide (Me_2SO) [31]. The same volume of solvent was added to the control samples to verify that the presence of Me_2SO had no effect. The uncoupler *p*-trifuoromethoxyphenylhydrazone of carbonylcyanide (FCCP) was kindly supplied by Dr Heytler of E. I. DuPont de Nemours Co. and was used as ethanolic solution. All other reagents were obtained from Sigma Chemical Co. Oligomycin was dissolved in 95 % ethanol.

RESULTS

From 10 types of aryl- and aralkylisothiocyanates with a different chemical structure, whose effects have been studied on the endogeneous respiration of Ehrlich-Lettré tumor cells (Miko, M. and Chance, B., in preparation), only BrPh-NCS, $(NCS)_2$-Ph_2 and Nap-CH_2NCS caused stimulation of the respiration of these cells. These substances differ markedly by their lipophilicity and also by their chemical reactivity (see Discussion). As can be seen from Fig. 1, BrPh-NCS stimulated respiration of tumor cells (Fig. 1A) in low concentrations. A general property of uncouplers of respiratory-chain phophorylation is that they inhibit respiration if added at concentrations greater than necessary for maximal uncoupling. This is demonstrated in Fig. 1B. The addition of oligomycin caused inhibition of endogeneous respiration of tumor cells (Fig. 2B). However, subsequent addition of 25 μM BrPh-NCS allowed respiration to proceed. Reversal of oligomycin-inhibited respiration by different concentrations of BrPh-NCS is linear in the range studied (Fig. 2A).

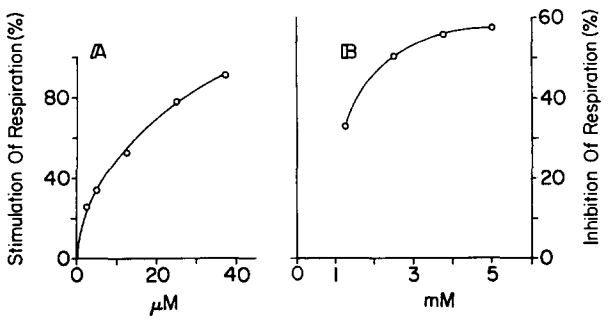

Fig. 1. The effect of *p*-bromophenylisothiocyanate on endogeneous respiration of Ehrlich-Lettré cells. 0.2 ml of cell suspension, containing 13.3 mg (A) and 16.4 mg (B) dry weight cells, were added to 2 ml of isotonic saline/phosphate medium pH 7.4 [24]. Oxygen uptake was measured at 30 °C. Rate of oxygen consumption by the samples with no BrPh-NCS was 135 nmol per min (A) and 114.5 nmol per min (B).

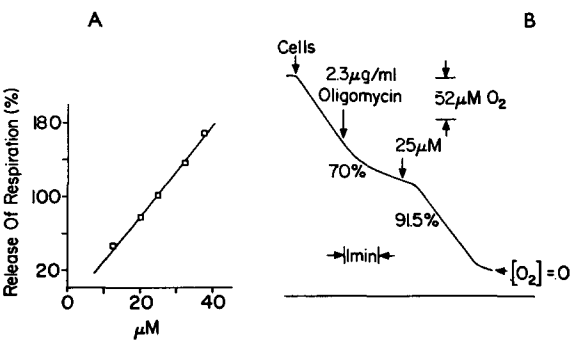

Fig. 2. The effect of *p*-bromophenylisothiocyanate on the respiration of oligomycin-inhibited ascites tumor cells. The cells were suspended at the final concentration 6.1 mg dry weight per ml. The control rate of oxygen uptake was 135 nmol per min. The other conditions were the same as for Fig. 1.

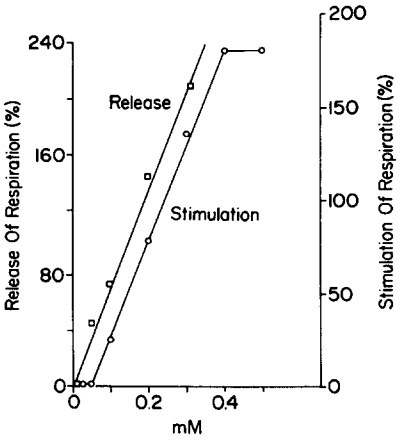

Fig. 3. Stimulation of Ehrlich-Lettré cell respiration and/or release of previously inhibited respiration of these cells by different concentrations of 4,4′-diisothiocyanatebiphenyl. The final concentration of the cells was 7.8 mg dry weight per ml. Oligomycin was added to a final concentration of 4.5 μg per ml. Control rate of oxygen consumption was 104 nmol per min.

$(NCS)_2$-Ph_2 stimulated endogeneous respiration of ascites tumor cells linearly from approximately 0.1 to 0.4 mM (Fig. 3). The maximal stimulation of respiration by $(NCS)_2$-Ph_2 required much higher concentrations than in the case of BrPh-NCS (approx. 10 times).

Fig. 4 shows the effect of Nap-CH_2NCS on the endogeneous respiration of ascites tumor cells. Up to 1 mM concentration Nap-CH_2NCS does not affect the respiration of the cells. Approx. 5 mM Nap-CH_2NCS is required for 50 % stimulation of respiration. The inhibition of respiration by oligomycin can be released by Nap-CH_2 NCS. 0.5 mM Nap-CH_2NCS is required for 50 % release of oligomycin inhibition of respiration.

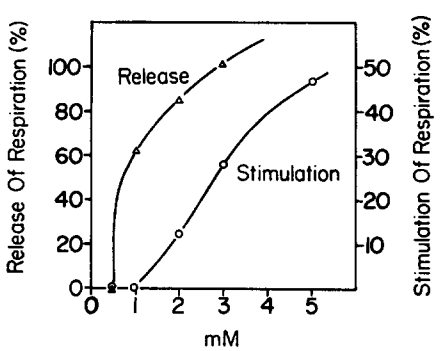

Fig. 4. Stimulation of Ehrlich-Lettré cell respiration and/or release of respiration previously inhibited by oligomycin by different concentrations of β-naphtylmethylisothiocyanate. The final concentration of the cells was 8.1 mg dry weight per ml. Oligomycin was added to a final concentration of 4.5 μg per ml. The control rate of oxygen consumption was 108 nmol per min.

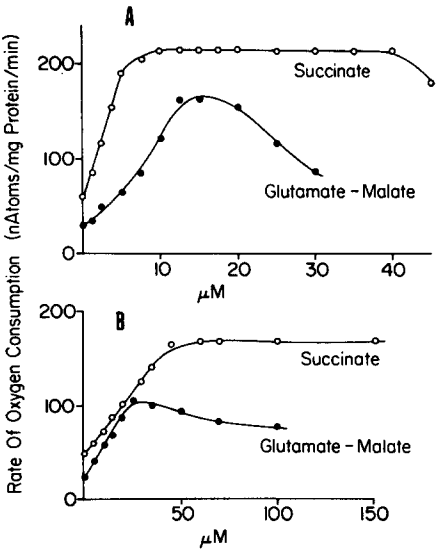

Fig. 5. The effect of *p*-bromophenylisothiocyanate on the rate of oxygen uptake by isolated pigeon heart (A) and rat liver mitochondria (B). The mitochondrial respiration was assayed in a medium containing 0.225 M mannitol, 0.075 M sucrose, 10 mM K_2HPO_4, 0.2 mM EDTA and 10 mM MOPS, pH 7.2. The mitochondria were suspended at 1.3 mg protein per ml (A) or 1.6 mg protein per ml (B). The substrates were 10 mM succinate in the presence of 3 μM rotenone or 5 mM glutamate plus 5 mM malate. The reaction temperature was 25 °C.

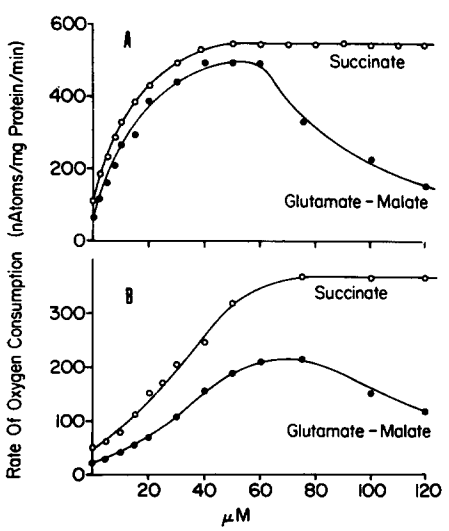

Fig. 6. The effect of 4,4′-diisothiocyanatebiphenyl on the rate of oxygen consumption by intact pigeon heart (A) and rat liver mitochondria (B). The mitochondria were suspended at 0.6 mg protein per ml (A) or 1 mg protein per ml (B). The other conditions were the same as for Fig. 5.

The effect of BrPh-NCS on succinate or glutamate plus malate oxidation by pigeon heart (A) and rat liver mitochondria (B) are compared in Fig. 5. The oxidation of both succinate or NAD-linked substrates measured in the absence of phosphate acceptor was stimulated by low concentrations of BrPh-NCS. For maximal activation of rat liver mitochondrial respiration by BrPh-NCS, higher concentrations were needed than in the case of pigeon heart mitochondria. As can be seen from Fig. 5, maximal stimulation of the oxidation of both succinate or NAD-linked substrates by pigeon heart mitochondria required approx. 15 μM BrPh-NCS.

$(NCS)_2$-Ph_2, similar to BrPh-NCS, stimulated respiration of both pigeon heart (A) and rat liver mitochondria (B). Maximal stimulation of mitochondrial respiration requires about 60 μM of $(NCS)_2$-Ph_2 for both mitochondria (Fig. 6), when either succinate or NAD-linked substrates were present. However, the rate of respiration per mg protein of pigeon heart mitochondria in the presence of isothiocyanates investigated is much higher than in the case of rat liver mitochondria. This might be explained by the higher concentration of cytochrome *a* per mg protein in pigeon heart mitochondria.

Nap-CH_2NCS requires a higher concentration than BrPh-NCS or $(NCS)_2$-Ph_2. When the substrate was succinate (pigeon heart mitochondria) maximal stimulation of respiration required approx. 400 μM of Nap-CH_2NCS (Fig. 7). When the substrates were glutamate plus malate, only a small stimulation of respiration of pigeon heart mitochondria followed by inhibition of respiration by higher concentrations. In rat liver mitochondria, the effect of Nap-CH_2NCS is similar to that observed in pigeon heart mitochondria, except that higher concentrations are required.

Mitochondrial respiration in the presence of ADP can be inhibited by oligo-

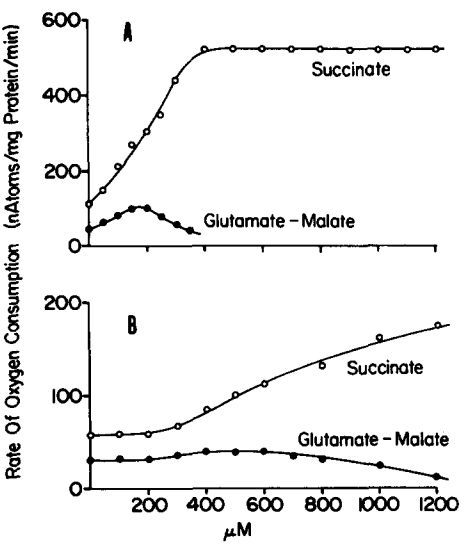

Fig. 7. The effect of β-naphtylmethylisothiocyanate on the rate of oxygen consumption by isolated pigeon heart (A) and rat liver mitochondria (B). The mitochondria were suspended at 0.6 mg protein per ml (A) or 1 mg protein per ml (B). The other conditions were the same as for Fig. 5.

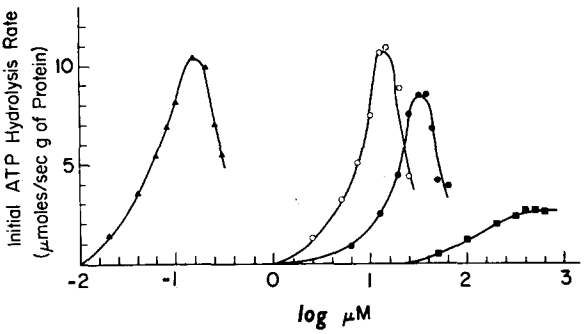

Fig. 8. Effects of FCCP (▲–▲), *p*-bromophenylisothiocyanate (○–○), 4,4′-diisothiocyanatebiphenyl (●–●) and β-naphtylmethylisothiocyanate (■–■) on the activation of mitochondrial ATPase. The rat liver mitochondria (0.36 mg per ml) were suspended to a final volume of 4 ml in a 0.12 M KCl and 0.02 M Tris · Cl medium (pH 7.1). The ATP was added to a final concentration of 1 mM. The reaction was started by adding rat liver mitochondria (25 °C). Control experiments gave an initial rate of hydrolysis of 0.64 μmol ATP per s per g of protein.

mycin [32, 33] and uncouplers of oxidative phosphorylation permit respiration to proceed in the presence of oligomycin. All studied isothiocyanates were capable of converting the inhibited mitochondria in state 3 to an uncoupled condition (results not shown). The observed reversal of oligomycin inhibition of respiration provides further evidence for the uncoupling activity of isothiocyanates.

Direct evidence of the uncoupling activity of isothiocyanates comes from measurements of ATPase activity (Fig. 8). ATPase activity of intact mitochondria is stimulated by isothiocyanates (BrPh-NCS and $(NCS)_2$-Ph_2) to nearly the same extent as by the uncoupler FCCP but higher concentrations of isothiocyantes are required.

DISCUSSION

Excessive concentrations of uncouplers of oxidative phosphorylation inhibit both mitochondrial respiration and the uncoupler-induced ATPase activity [34–39]. These inhibitory effects have been studied in a number of laboratories. Isothiocyanates have been found both to activate and to inhibit oxidation. However, in the case of BrPh-NCS and other aromatic isothiocyanates, the respiratory inhibition could be due to an inhibition of several dehydrogenases. Drobnica [40, 41] found that BrPh-NCS inhibited succinate-, glutamate- and glucose-6-phosphate dehydrogenases, especially glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12). Daehnfeld [23] observed a decrease of the total oxygen uptake by 20–45 % in the presence of isothiocyanate esters and succinate dehydrogenase, glucose-6-phosphate dehydrogenase, and the oxidation of NADH by mitochondria from Ehrlich's ascites tumor cells found to be inhibited. Hasilik and Livar [42] found that activity of NADH-cytochrome *c* oxidoreductase and NADH oxidase is markedly decreased in the mitochondrial fraction prepared from *Candida albicans* cells incubated with a lower dose of BrPh-NCS. The same conditions resulted in a significant but smaller inhibition of succinate and cytochrome *c* oxidase.

Maximal activation of a latent mitochondrial ATPase activity of rat liver mitochondria in the presence of BrPh-NCS was found at concentration of 15 μM (Fig. 8). Kraayenhof [43] measured 2,4-dinitrophenol-induced ATPase of rat liver mitochondria at different ATP and uncoupler concentrations. In agreement with previous results, higher concentrations of dinitrophenol have an inhibitory effect.

The investigated isothiocyanates differ significantly in their solubility in organic solvents and in their chemical reactivity. The values of partition coefficients for the system octanol/water (25 °C) were determined: log P = 4.03 for BrPh-NCS, 5.5 for $(NCS)_2$-Ph_2 and 4.42 for Nap-CH_2NCS respectively. The values of the rate constants of nucleophilic reaction with glycine were k glycine (25 °C) = 21.1 for BrPh-NCS, 14.9 for $(NCS)_2$-Ph_2 and 7.64 $M^{-1} \cdot min^{-1}$ for Nap-CH_2NCS respectively [17, 44]. These data make it possible to conclude that only in the case of BrPh-NCS was stimulation of respiration of tumor cells, as well as the maximum release by oligomycin-inhibited respiration, obtained by concentrations which did not pass the solubility limit in the given buffer. It is interesting to note that in the case of BrPh-NCS the inhibition of cell respiration was obtained only by concentrations (1–5 mM) much higher than the solubility limit in water (54 μM). In the next two derivatives the inhibition of cell respiration was not observed even with concentrations much higher than the solubility in water (25 °C). We assume that the greater the partition coefficient in a series of isothiocyanates grouped according to the increasing value of log P, the greater will be their uncoupling activity, but only up to a certain degree. Any further increase of log P will be marked by a decrease of this activity. By using a richer series, especially isothiocyanates which are similar by their chemical structure, it will be possible to "designate" the value of the optimum partition coefficient. It concerns both the stimulating effect on the respiration of whole cells and also the uncoupling activity of mitochondria. Isothiocyanate reactivity is over 1000 times higher towards -SH groups than the reactivity towards amino groups [44]. Therefore, if the uncoupling effect was the expression of the applicability of isothiocyanates, then only the chemical reaction with -SH protein groups could come into consideration.

Drobnica et al. [45, 46] recently investigated the effects of various thiol-combining agents on protein and non-protein -SH groups and on succinate oxidation of rat liver mitochondria. Under their conditions, the SH content of rat liver mitochondria was found to be around 90 nmol SH per mg protein. *p*-Bromophenylisothiocyanate, in concentrations of 30–300 nmol per mg protein of rat liver mitochondria, reacts only with non-protein SH groups and causes complete loss of respiratory control. The non-protein SH content of rat liver mitochondria is represented by reduced glutathione (> 95 %). The experiments with ^{35}S labelled BrPh-NCS and other mono- and polynuclear aromatic isothiocyanates indicated that their main part is bound to the isolated mitochondria.

As we have indicated elsewhere, in a brief report [47], a more direct measure of uncoupling properties of isothiocyanates is seen in Fig. 8, where the effects on the rate of ATP hydrolysis are shown. These results are in good agreement with those obtained by Miko and Drobnica (Miko, M. and Drobnica, L., unpublished). BrPh-NCS, $(NCS)_2$-Ph_2 and Nap-CH_2NCS decreased the level of ATP in rat liver mitochondria with succinate or glutamate plus malate as substrates. A paper dealing with the mechanism of the uncoupling effect of *p*-bromophenylisothiocyanate and other new SH-combining agents will be published elsewhere.

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REFERENCES

1 Antoš, K., Drobnica, L. and Kováč, Š., eds, (1964) Proc. 1st Int. Symp. Isothiocyanates, Slovak Polyt. Univ. Press, Bratislava
2 Antoš, K., Drobnica, L. and Kováč, Š., eds, (1969) Proc. 2nd Int. Symp. Isothiocyanates, Slovak Polyt. Univ. Press, Bratislava
3 Drobnica, L., Zemanová, M., Antoš, K., Kristián, P., Martvoň, A. and Závodská, E. (1968) Appl. Microbiol. 16, 582–587
4 Drobnica, L., Augustin, J., Svoboda, A. and Nečas, O. (1968) Can. J. Microbiol. 14, 853–860
5 Nemec, P., Drobnica, L., Ondrejičková, O. and Odlerová, Ž. (1970) in Proc. 6th Int. Congr. Chemother., Vol. 1, pp. 125–128, University of Tokyo Press, Tokyo
6 Nemec, P., Drobnica, L., Augustin, J., Odlerová, Ž. and Ondrejičková, O. (1972) in Proc. 7th Int. Congr. Chemother., Vol. 1, pp. 11/34–38, Urban and Schwarzenberg, München
7 Augustin, J., Drobnica, L., Nemec, P., Antoš, K., Kristián, P., Martvoň, A. and Uher, M. (1968) J. Antibiot. 21, 504–508
8 Horáková, K., Drobnica, L. and Nemec, P. (1971) Neoplasma 18, 355–359
9 Miko, M. and Ujházy, V. (1968) Neoplasma 15, 357–363
10 Miko, M. and Drobnica, L. (1969) Folia Fac. Med. Univ. Comenianae Bratisl. 7, 217–285
11 Rada, B., Hanušovská, T., Drobnica, L. and Nemec, P. (1971) Acta Virol. 15, 329–332
12 Vlachová, D. and Drobnica, L. (1968) Collect. Czech. Chem. Commun. 31, 997–1008
13 Lien, E. J., Hansch, C. and Anderson, M. S. (1968) J. Med. Chem. 11, 430–443
14 Nemec, P., Drobnica, L., Kristián, P., Antoš, K. (1963) Folia Microbiol. Prague 8, 187–193
15 Drobnica, L., Zemanová, M., Nemec, P., Antoš, K., Kristián, P., Štullerová, A., Knoppová, V. and Nemec, Jr, P. (1967) Appl. Microbiol. 15, 701–709
16 Drobnica, L. and Nemec, P. (1969) in Proc. 2nd Int. Symp. Isothiocyanates (Antoš, K., Drobnica, L. and Kovác, Š., eds), pp. 67–71, Slovak Polyt. Univ. Press, Bratislava
17 Drobnica, L. and Augustin, J. (1965) Coll. Czech. Chem. Commun. 30, 1618–1625
18 Drobnica, L., Augustin, J., Ondrejičková, O. and Nemec, P. (1972) Abstr. Commun. Meet. FEBS Vol. 8, p. 461, North-Holland, Amsterdam
19 Drobnica, L., Nemec, P., Antoš, K., Kristián, P. and Hulka, A. (1963) in Proc. 5th Int. Congr. Biochem. (Sissakian, A. N. M., ed.), Vol. 5, pp. 6–9, Pergamon Press, Oxford
20 Drobnica, L. (1967) in Wirkungsmechanismen von Fungiziden und Antibiotika (Gibard, E., ed.), pp. 131–139, Akademie Verlag, Berlin
21 Drobnica, L. (1967) in Wirkungsmechanismen von Fungiziden und Antibiotika (Gibard, E., ed.), pp. 404–408, Akademie Verlag, Berlin
22 Drobnica, L., Klenow, H. and Betina, V. (1965) Abstr. Commun. Meet. FEBS Vol. 2, p. 321, Vienna
23 Daehnfeldt, J. L. (1968) Biochem. Pharmacol. 17, 511–518
24 Chance, B. and Hess, B. (1959) J. Biol. Chem. 234, 2404–2412
25 Miko, M. and Chance, B. (1975) FEBS Lett. in press
26 Chance, B. and Hagihara, B. (1963) in Proc. 5th Int. Congr. Biochem. (Sissakian, A. N. M., ed.) Vol. 5, pp. 3–37, Pergamon Press, Oxford
27 Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) J. Biol. Chem. 177, 751–766
28 Chance, B. (1959) in Ciba Foundation Symposium on Cell Metabolism (Wolstenholme, G. E. and O'Connor, eds), pp. 91–129, Little Brown and Co., Boston
29 Wilson, D. F. (1969) Biochemistry 8, 2475–2488
30 Nishimura, M., Ito, Z. and Chance, B. (1962) Biochim. Biophys. Acta 59, 177–186
31 Drobnica, L., Augustin, J. and Miko, M. (1970) Experientia 26, 506–508
32 Racker, E. (1965) Mechanisms in Bioenergetics, pp. 155–170, Academic Press, New York

33 Lardy, H. A., Connely, J. L. and Johnson, D. (1964) Biochemistry 3, 1961–1968
34 Chance, B., Williams, G. R. and Hollunger, G. (1963) J. Biol. Chem. 238, 439–444
35 Chance, B. and Hollunger, G. (1963) J. Biol. Chem. 238, 445–448
36 Papa, S., Lofrumento, N. E., Paradies, G. and Quagliariello, E. (1968) Biochim. Biophys. Acta 153, 306–308
37 Wislon, D. F. and Merz, R. D. (1967) Arch. Biochem. Biophys. 119, 470–476
38 Wilson, D. F. and Merz, R. D. (1969) Arch. Biochem. Biophys. 129, 79–85
39 van Dam, K. and Slater, E. C. (1967) Proc. Natl. Acad. Sci. U.S. 58, 2015–2020
40 Drobnica, L. (1960) Neoplasma 7, 385–391
41 Drobnica, L. (1964) Folia Microbiol. Prague 9, 182–183
42 Hasilik, A. and Livar, M. (1971/72) Chem.-Biol. Interactions 4, 305–310
43 Kraayenhof, R. (1971) Uncoupling of Energy Conservation in Chloroplast and Mitochondrion, Ph.D Thesis, pp. 60–76, Mondeel-Offsetdrukkerij, Amsterdam
44 Drobnica, L. (1970) Isothiocyanates as Biological Active Substances. DSc. Thesis, pp 25–89, Slovak Polyt. University, Bratislava
45 Drobnica, L., Chance, B. and Scarpa, A. (1974) Abstr. Commun. Meet. FEBS Budapest, Vol. 9, p. 282, North-Holland, Amsterdam
46 Drobnica, L., Miko, M. and Nemec, P. (1975) 5th Colloquium on Bioenergetics and Mitochondria, Varna, September 15–21, submitted for publication.
47 Miko, M. and Chance, B. (1973) Abstr. Commun. 9th Int. Congr. Biochem., Stockholm, p. 220