more likely explaination might be that Cd^{2+} at concentrations of 20 and 200 μM stabilized the lysosomal membrane, inhibiting the fusion between lysosomes and phagosomes [13], and hence proteolysis. Indeed, a stabilizing effect of Cd^{2+} on lysosomal membranes has been reported [3].

Degradation by lysosomes requires energy either for an ATP-driven proton pump necessary for the maintainance of the intralysosomal acidity [14-16], and/or, for other processes involved in the catabolic pathways [17]. Hence, uncoupling of the oxidative phosphorylation by Cd^{2+} [11, 12] may also explain the observed inhibitory effect of Cd^{2+} on the degradation of ^{125}I-HSA.

Cd^{2+} might also inhibit lysosmal proteases, but probably only at extremely high concentrations [4].

In conclusion, the immediate inhibition of ^{125}I-HSA degradation by Cd^{2+} is most likely caused by a combination of membrane stabilization and inhibition of the oxidative phosphorylation.

Drugs usually classified as membrane stabilizing agents can also labilize membranes *in vitro* at extremely high concentrations [18–21]. Accordingly, the observed stabilization of lysosomal membranes by Cd^{2+} [3] could at extremely high concentrations (2000 μM) be replaced by labilization.

The experimental system described here should be a useful tool in the study of the endocytic and degradative activity of NPC. The number of drugs that may be used is large, and the conditions for *in vitro* incubation are readily controlled.

Acknowledgements—I am indebted to Dr. Trond Berg for his advice and careful reading of the manuscript. Mrs. Turid Berdal Gangnæs gave skilful technical assistance.

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REFERENCES

1. J. L. Mego, *Lysosomes in Biology and Pathology* (Eds. J. T. Dingle and H. B. Fell) Vol. 3, pp. 527–537. North Holland, Amsterdam (1973).
2. M. Nilsson and T. Berg, *Biochim. biophys. Acta.* **497**, 171 (1977).
3. M. Chvapil, J. N. Ryan and Z. Brada, *Biochem. Pharmac.* **21**, 1097 (1972).
4. J. L. Mego and J. A. Cain, *Biochem. Pharmac.* **24**, 1227 (1975).
5. J. L. Mego, F. Bertini and D. Mc Queen, *J. Cell Biol.* **32**, 699 (1967).
6. T. Berg and J. Mørland, *Biochim. biophys. Acta.* **392**, 233 (1975).
7. P. O. Segelen, *Methods in Cell Biology* (Ed. D. M. Prescott) Vol. 13, pp. 29–83. Academic Press, New York (1975).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
9. Z. A. Shaikh and O. J. Lucis, *Archs Environ. Hlth* **24**, 410 (1972).
10. A. Zanvil and M. D. Cohn, *J. exp. Med.* **124**, 557 (1966).
11. A. Sporn, I. Dinu, L. Stonescu and A. Cirstea, *Nahrung* **13**, 461 (1969).
12. E. E. Jacobs, M. Jacob, D. R. Sandai and L. B. Bradley, *J. biol. Chem.* **223**, 147 (1956).
13. C. de Duve, in *The Interaction of Drugs and Subcellular Components in Animal Cells* (Ed. P. N. Campbell), pp. 155–169. J. & A. Churchill, London (1968).
14. J. L. Mego and R. M. Farb, *Biochem. J.* **172**, 233 (1978).
15. P. Dell'Antone, *Biochem. biophys. Res. Commun.* **86**, 180 (1979)
16. J. L. Mego, *Fedn. Eur. biochem. Soc. Lett.* **107**, 113 (1979).
17. A. Hollermans, D-J. Reijngoud and J. M. Tager, *Biochim. biophys. Acta.* **551**, 55 (1979).
18. P. S. Seeman, *Biochem. Pharmac* **15**, 1632 (1966).
19. P. S. Seeman and J. Weinstein, *Biochem. Pharmac* **15**, 1737 (1966).
20. P. S. Seeman, *Biochem. Pharmac.* **15**, 1753 (1966).
21. P. S. Seeman, *Biochem. Pharmac.* **15**, 1767 (1966).

Biochemical Pharmacology, Vol. 29, pp. 3105–3109.

0006-2952/80/1115-3105 $02.00/0

Effects of salicylate–copper complex on the metabolic activation in phagocytizing granulocytes

(*Received* 6 *December* 1979; *accepted* 1 *July* 1980)

The uptake of particles by human polymorphonuclear leucocytes (PMN) is associated with a strong increase in oxygen consumption [1–3] and with a concomitant generation of toxic oxygen metabolites such as superoxide anion (O_2^-) [4], hydrogen peroxide (H_2O_2) [5] and hydroxyl free radical (OH^-) [6]. Under normal conditions these toxic metabolites appear to promote the killing of bacteria in PMN [7, 8]. Moreover, immune complexes and aggregated immunoglobulin G also induce PMN to elaborate O_2^- and H_2O_2 [9]. The question arises as to whether this phenomenon causes a major part of PMN-mediated tissue injury that occurs with inflammation. Accordingly, a number of compounds endowed with anti-inflammatory activity interfere with the altered oxygen metabolism that accompanies the phagocytic process [12]. Recently, Sorenson [13] reported that salicylate–copper complex (Cu(II)–Sal_2) has stronger anti-inflammatory activity than salicylate alone, which suggests that chelate might be the active form of the drug. In view of this it is of interest to obtain information about the effects of Cu(II)–Sal_2 on oxygen-dependent PMN metabolism as compared with those induced by salicylate alone. We have therefore investigated the effects of salicylate and Cu(II)–Sal_2 on phagocytosis-induced PMN metabolic activation (measured by zymosan-stimulated oxygen consumption, NBT reduction and iodination) compared to the effects of these compounds on the extent of phagocytosis (measured by the ingestion rate of *Klebsiella pneumonie*).

Materials and methods

Chemicals. Zymosan A from *Saccharomyces cerevisae*, nitroblue tetrazolium (NBT) and superoxide dismutase were obtained from Sigma Chemical Co., St. Louis, MO,

U.S.A. The Dextran was from Pharmacia, Uppsala, Sweden. Soluene-350 was from Packard Inst. Co., Downers Grove, IL, U.S.A.

Apparatus. Copper content of the complex was measured with a Perkin–Elmer atomic absorption spectrophotometer model 300. Oxygen tension was determined polarographically using a Gilson oxygraph equipped with a Clark electrode, from Yellow Springs Inst.

Preparation of the copper complex. The complex was prepared as described in Ref. 14 with minor modifications. $CuSO_4$ solution ($CuSO_4$, $5H_2O$ 12.5 g/200 ml water) was added to a sodium salicylate solution (16 g/100 ml water). After cooling for a few minutes in an ice bath, blue-green crystals were precipitated. The precipitate was removed and dissolved again in 50 ml boiling water. Recooling resulted in renewed crystallization of the complex. Measurement of the copper content of the complex by atomic absorption according to a standard assay procedure [15] indicated that there was one copper cation for every two molecules salicylates.

Isolation of leucocytes. Human PMN were isolated from heparinized venous blood by sedimentation of the erythrocytes using Dextran as previously described [16]. The final pellet was resuspended in calcium-free Krebs–Ringer phosphate buffer (KRP) pH 7.40 containing 5.5 mM glucose and 10% AB serum. Isolated cells were adjusted to a concentration of 10^7 PMN/ml. Aliquots of this suspension were used in each experiment.

Patients. The CGD patients were three males ranging in age from 2 to 7. The diagnosis of the X-linked CGD was established by measuring oxygen consumption and quantitative NBT reduction in zymosan-stimulated PMN in the patients' mothers. There was about a 50 per cent decrease as expected for X-linked CGD.

PMN function tests. (1) Ingestion rate of bacteria was measured by a modified Mandell's technique [17] as follows: Heat-killed non-virulent ^{14}C-labelled bacteria (*Klebsiella pneumoniae*) after opsonization in AB serum were selected by differential centrifugation. Low weight bacteria which did not sediment at 350 $g \times 8$ min (4°) were suspended in KRP at a concentration of 2×10^8 bacteria/ml 0.5 ml of suspended PMN (5×10^5 cells/ml) and 0.5 ml of suspended bacteria (5×10^7 bacteria/ml) were mixed in plastic tubes. Before (O') and after tumbing the tubes (20 rpm) for 10 min, the ingestion process was stopped in ice-cold acid–citrate–dextrose. Separation of non-ingested bacteria from the PMN was performed by three centrifugations and washing at 150 $g \times 6$ min. The cell pellets were dissolved with soluene-350 and counted in an Intertechnique (ABAC SL 40) scintillation counter. The specific radioactivity of labelled bacteria was determined in each experiment so that the selective radioactivity could be directly converted to number of bacteria ingested by the PMN. The results were expressed in terms of bacteria associated per PMN in 10 min at 37°.

(2) Oxygen consumption of zymosan-stimulated PMN was measured polarographically according to Kvarstein [18]. Incubation medium was composed of KRP, 5.5 mM glucose, 10% AB serum and 10^6 PMN/ml. The results are expressed in nmoles of oxygen consumed per min and per 10^6 PMN at 37°.

(3) Zymosan-stimulated NBT reduction was measured according to Baehner and Nathan [19] except that cyanide was omitted. The incubation time was 15 min at 37° and the results were expressed in nmoles NBT reduced per min and per 10^6 PMN.

(4) The iodination test was performed at 37° according to Pincus and Klebanoff as modified by Hakim *et al.* [16]. The incubation medium contained 10% AB serum and 20 μM iodide in KRP. Incubation times were 0, 10 and 20 min. The results, expressed in nmoles iodide converted to a trichloracetic precipitable form per min and per 10^7 PMN, were the means of the results for both incubation times.

Treatment of PMN with Cu(II)–*Sal*$_2$. Cu(II)–Sal_2 dissolved in dimethylsulfoxide (DMSO) was diluted in distilled water (1/50, v/v). This solution, containing various concentrations of Cu(II)–Sal_2, was added (2/100, v/v) to KRP, containing about 10^7 PMN/ml. After incubation, the PMN were centrifuged, washed, resuspended in KRP and counted again. Control experiment PMN were identically treated, except that Cu(II)–Sal_2 was not present in DMSO.

Results

Effects of Cu(II)–Sal_2 *complex and salicylate on the PMN zymosan stimulated oxygen consumption at various preincubation times.* In phagocytizing PMN, the increase in oxygen consumption reflects the activation of the oxidative system which catalyses the reduction of molecular oxygen to O_2^- and H_2O_2 in the presence of NAD(P)H. There is a considerable evidence that this system is located in plasma and/or granule membranes of the cells (for general review see Ref. 20). Small hydrophobic molecules such as the Cu(II)–Sal_2 complex may successfully reach the active site of the membrane-bound system. However, the time required for the complex to arrive at the active site of the system must be carefully taken into consideration in the experiments. Accordingly, preliminary investigations indicate that the preincubation times of PMN in a medium containing Cu(II)–Sal_2 complex is determinant in the development of the complex's effect on the PMN metabolic response associated with phagocytosis. Results reported in Fig. 1 indicate that the addition of 50 μM of complex just before the addition of zymosan does not affect stimulated oxygen uptake, whereas increasing preincubation time results in a strong inhibition of stimulated oxygen uptake. Under similar experimental conditions, salicylate does not affect oxygen uptake.

Effects of varying concentrations of Cu(II)–Sal_2 *complex and salicylate on the PMN zymosan-induced metabolic activation.* Besides oxygen consumption, quantitative NBT reduction and iodination are other tests widely used to determine the extent of metabolic activation associated with phagocytosis in PMN. Preincubation of PMN in the

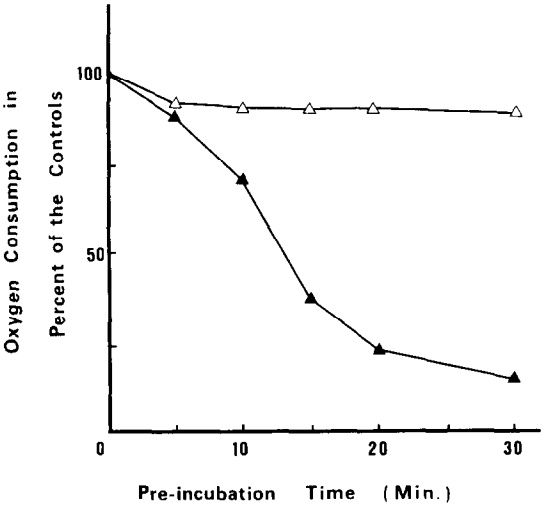

Fig. 1. Effect of the preincubation time of Cu(II)–Sal_2 and salicylate with PMN on the zymosan-stimulated oxygen consumption. Time 0 indicates that either Cu(II)–Sal_2 or salicylate was added to the medium just before the addition of zymosan. ▲——▲ indicates Cu(II)–Sal_2 addition and △——△ salicylate addition. All results were the mean ± S.D. of six different experiments. Value of the control (100%) was 14.82 ± 1.86 nmoles/min/10^6 PMN of oxygen consumed.

presence of various concentrations of Cu(II)–Sal_2 results in a strong inhibition of oxygen uptake, NBT reduction and iodination. Under similar conditions salicylate exhibits no effect. Experimental data shown in Fig. 2 show that the extent of inhibition is linear with respect to the Cu(II)–Sal_2 log. concentration for oxygen uptake and iodination whereas results shown in Fig. 3 show that the log. of NBT reduction is linear with respect to the Cu(II)–Sal_2 log. concentration, which indicates the highly inhibitory activity of the complex. If ID_{50} is the concentration of the complex which reduces the biochemical response associated with phagocytosis by 50 per cent, the average of ID_{50} is 25 μM for the inhibition of oxygen uptake, 40 μM for the inhibition of iodination and 9 μM for the inhibition of NBT reduction.

Effects of varied concentrations of Cu(II)–Sal_2 *complex and salicylate on phagocytosis.* It is generally agreed that in normal phagocytizing PMN, the extent of metabolic activation depends on the number of particles engulfed [21, 22]. Thus, the inhibitory effect of Cu(II)–Sal_2 on zymosan-stimulated oxygen uptake could be due to an inhibition of the phagocytic process. In order to test this possibility, we have determined the ingestion rate of bacteria by PMN in experimental situations similar to those used for measuring the metabolic activation of the cells. Results of these experiments have indicated that neither Cu(II)–Sal_2 nor salicylate interferes with phagocytosis to any extent since the number of bacteria associated with PMN was found to be 6.8 ± 2.6 (number of bacteria ± S.D. associated per PMN) in the control experiments, 7.8 ± 1.4 in PMN preincubated 15 min in the presence of 100 μM salicylate and 6.0 ± 2.3 in PMN preincubated 15 min in the presence of 100 μM Cu(II)–Sal_2.

PMN function tests in cytotoxic capability deficient cells. Under certain experimental conditions, as well as in various diseases, PMN fail to promote the killing of some bacteria. Such is the case when superoxide dismutase (SOD) is added to the assay media of functional tests [7] or when PMN are isolated from patients with chronic granulomatous disease (CGD), a hereditary disorder characterized by acute susceptibility to infection [23, 24]. PMN from patients with CGD effectively ingest microorganisms but their metabolic activation is deficient [25]. This abnormality results in an inability to kill bacteria and fungi [26]. In both cases, PMN can be considered toxic capability deficient cells. From the point of view of the present report, it is of interest to determine the engulfment of bacteria and the metabolic response associated with phagocytosis of PMN from patients with CGD or in the presence of exogenous SOD. Results summarized in Table I indicate that treatment of normal PMN with Cu(II)–Sal_2 creates a model of CGD whereas addition of SOD slightly affects the functional tests of PMN. However, a significant inhibition of NBT reduction and oxygen consumption in the presence of SOD should be pointed out. The extent of inhibition of NBT reduction is consistent with an artifact as previously reported [27] while the apparent inhibition of oxygen consumption remains unexplained.

Discussion

Polymorphonuclear leucocytes are the predominant cells in the loci of inflammation and the release of toxic oxygen metabolites by these cells is one of the postulated causes of tissue damage at inflammatory sites [9–11]. There is evidence of the release of toxic oxygen metabolites from PMN during phagocytosis [4–6] as well as from cells stimulated by immune complexes [9]. In both cases, the generation of toxic oxygen species is the result of an increase of the oxidative metabolism of the cells. The extent of this metabolic activation may be quantified with accuracy by measuring various parameters such as oxygen uptake, NBT reduction and iodination. These tests are considered good windows into the cytotoxic potential of these cells. Accordingly, PMN from patients with chronic granulomatous disease who have acutely impaired bactericidal activity fail to increase oxygen consumption, NBT reduction and iodination during phagocytosis. Experimental data reported in this paper show that PMN treated with Cu(II)–Sal_2 behave in a way similar to PMN in patients with CGD, i.e. normal phagocytizing activity associated with a defect in metabolic activation. In this case, it is reasonable to think that Cu(II)–Sal_2 complex sharply decreases the toxic potential of PMN, and that this phenomenon is responsible for the anti-inflammatory activity of the complex. It is interesting to note that in similar experimental situations, salicylate exhibits no inhibitory effect on PMN metabolic activation. If a relationship exists between the ability of anti-inflammatory drugs to inhibit the metabolism of PMN and their pharmacological properties, these results suggest that the copper chelate is in fact an active form of salicylate. However, it remains to be determined whether salicylate copper complex is found *in vivo*. The mechanism by which the complex affects the oxidative metabolism of the PMN is not elucidated. However, it must be pointed out that Cu(II)–Sal_2 exhibits a scavenging effect on O_2^- by a SOD-like activity [14]. This means that the complex may react with free O_2^- as well as with oxygen carried in a superoxide state as observed in the superoxo-ferriheme structure (peroxidase–FeIII–O_2^- of peroxidase) [28]. Moreover, this redox property indicates that the complex may react with various components of the electron transport chain, especially with a compound such as cytochrome *b* which was recently suggested to be involved in the oxidative metabolism of

Table 1. Comparative studies of the functional activities of normal PMN under control conditions, in the presence of SOD, preincubated with Cu(II)–Sal_2 and the functional activities of PMN from patients with X-linked CGD*

	Ingestion rate of *Klebsiella*	Oxygen uptake	NBT reduction	Iodination
Control subjects	6.8 ± 2.6	14.82 ± 1.86	1.78 ± 0.36	15.80 ± 4.60
Control subjects + SOD	7.2 ± 2.0	(11.20 ± 2.01)†	(1.42 ± 0.20)†	15.98 ± 4.10
Control subjects +Cu(II)–Sal_2	5.9 ± 3.0	(0.00)†	(0.00)†	(1.60 ± 0.32)†
Patients with CGD	(9.4 ± 3.1)†	(0.00)†	(0.00)†	(0.00)†

* Where indicated, SOD was added at a concentration of 320 nM and Cu(II)–Sal_2 at a concentration of 100 μM with a 30 min preincubation. Except for the CGD PMN study, all results were the means ± S.D. of six different experiments.

† Statistically significant values which differed from the controls within a 5% significance limit.

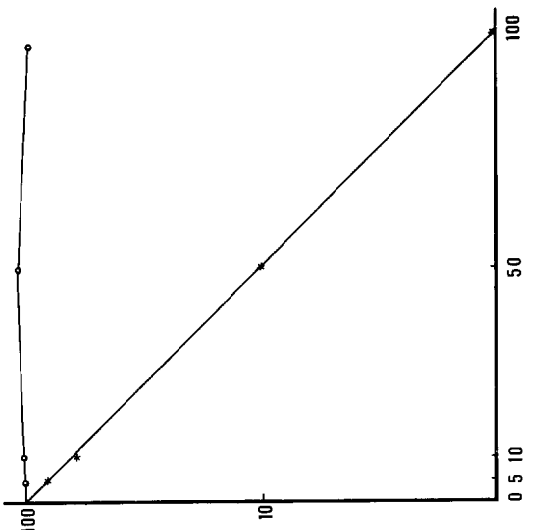

Fig. 3. Effects of various concentrations of Cu(II)–Sal_2 and salicylate on the quantitative NBT reduction. Quantitative NBT reduction was measured as described in Methods, where PMN were preincubated with Cu(II)–Sal_2 or salicylate for 15 min. ○——○ indicates data obtained with salicylate and ●——● data obtained with Cu(II)–Sal_2. Value of the control ± S.D. was 1.78 ± 0.36 nmoles/min/10^6 cells of NBT reduced. All values were the means of six different experiments.

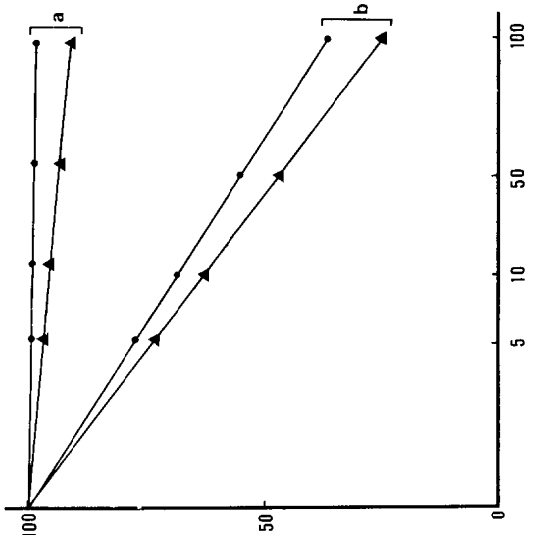

Fig. 2. Effects of various concentrations of Cu(II)–Sal_2 and salicylate on PMN zymosan-stimulated oxygen consumption and iodination. Oxygen consumption and iodination were measured as described in methods where PMN were preincubated with Cu(II)–Sal_2 or salicylate for 15 min. ▲——▲ indicates oxygen consumption and ●——● iodination. (a) indicates data obtained with salicylate, and (b) those obtained with Cu(II)–Sal_2. Values of the controls ± S.D. were: zymosan stimulated oxygen consumption 14.82 ± 1.86 nmoles/min/10^6 cells oxygen consumed and iodination 15.80 ± 4.60 nmoles/hr/10^7 cells of iodide converted to a trichloracetic precipitable form. All values were the means of six different experiments.

PMN [29]. SOD itself has been found to be anti-inflammatory [10]. This property has been related to a scavenging effect on O_2^- and a protective effect against the autoxidation of stimulated PMN [11]. Our findings regarding the effect of SOD on the functional activity of PMN show that PMN behave quite differently in the presence of SOD as compared to PMN treated with Cu(II)–Sal_2. This could mean that SOD, due to its relatively high molecular weight, remains outside the cells and does not interfere with the oxidative system of the cells, whereas the complex's small size favours its permeability across biological membranes. Consequently it reaches the active site of the oxidative system of PMN. Observations concerning the lag time necessary for the complex to inhibit the respiratory burst in PMN support this assumption. An exact determination of the enzymatic system, sensitive to the inhibitory effect of the complex as well as its intracellular localization, will help to understand oxidative metabolism in PMN.

In summary, phagocytosis-induced metabolic activation in human polymorphonuclear leucocytes has been found to be strongly inhibited by salicylate–copper complex (Cu(II)–Sal_2) whereas under similar experimental conditions salicylate exhibits no effect. These results are in agreement with the hypothesis suggesting that copper chelate is an active form of the drug.

Acknowledgement—This work was supported by a grant of INSERM (CRL 79.5.010.2)

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REFERENCES

1. C. W. Baldridge and R. W. Gerard *Am. J. Physiol.* **103**, 235 (1933).
2. Z. A. Cohn and S. L. Morse *J. exp. Med.* **111**, 667 (1960).
3. A. J. Sbarra and M. L. Karnovsky *J. biol. Chem.* **234**, 1355 (1962).
4. B. M. Babior, R. S. Kipnes and J. T. Curnutte, *J. clin. Invest.* **52**, 741 (1973).
5. B. Paul and A. J. Sbarra *Biochim. biophys. Acta* **156**, 168 (1968).
6. A. I. Tauber and B. M. Babior, *J. clin. Invest.* **60**, 374 (1977).
7. R. B. Johnston, B. B. Keele, M. P. Misra, J. E. Lehmeyer, L. S. Weeb, R. L. Baener and K. V. Rajagopalan, *J. clin. Invest.* **55**, 1357 (1975).
8. R. B. Johnston and J. E. Lehmeyer, In *Superoxide and Superoxide Dismutases* (Eds. A. M. Michelson, J. M. McCord and I. Fridovich), p. 291. Academic Press, New York (1977).
9. R. B. Johnston and J. E. Lehmeyer, *J. clin. Invest.* **57**, 836 (1976).
10. J. M. McCord, *Science* **185**, 529 (1974).
11. M. L. Salin and J. M. McCord, *J. clin. Invest.* **56**, 1319 (1975).
12. M. J. Cline, *N. Engl. J. Med.* **28**, 1187 (1974).
13. J. R. Sorenson, *J. med. Chem.* **19**, 135 (1976).
14. L. R. De Alvare, K. Goda and T. Kimura, *Biochem. biophys. Res. Commun.* **69**, 687 (1976).
15. E. Berman, *Atomic Absorption News Lett.* **4**, 296 (1965).
16. J. Hakim, E. Cramer, P. Boivin, H. Troubé and J. Boucherot, *Eur. J. clin. Invest.* **5**, 215 (1975).
17. G. L. Mandell, *J. clin. Invest.* **55**, 561 (1975).
18. B. Kvarstein, *Scand. J. clin. Lab. Invest.* **25**, 337 (1970).
19. R. L. Baehner and D. G. Nathan, *N. Engl. J. Med.* **278**, 971 (1968).
20. M. L. Karnovsky, in *The Phagocytic Cell in Host Resistance* (Eds. J. A. Bellanti and D. H. Dayton) p. 25. Raven Press, New York (1975).
21. T. P. Stossel, *N. Engl. J. Med.* **28**, 717 (1974).
22. M. Torres, D. de Prost, J. Hakim and M. A. Gougerot, *Eur. J. clin. Invest.* **9**, 209 (1979).
23. R. A. Bridges, H. Berendes and R. A. Good, *Am. J. Dis. Child.* **97**, 387 (1959).
24. B. Holmes, P. G. Quie, D. B. Windhorst and R. A. Good, *Lancet* **1**, 1225 (1966).
25. B. Holmes, A. R. Page and R. A. Good, *J. clin. Invest.* **46**, 1422 (1967).
26. P. G. Quie, J. G. White, B. Holmes and R. A. Good, *J. clin. Invest.* **46**, 668 (1967).
27. C. Auclair, M. Torres and J. Hakim, *Fedn. Eur. biochem. Soc. Lett.* **89**, 26 (1978).
28. T. Odajima, *Biochim. biophys. Acta* **235**, 52 (1971).
29. A. W. Segal and O. T. G. Jones, *Nature Land.* **276**, 515 (1978).

Biochemical Pharmacology, Vol. 29, pp. 3109–3112.

0006-2952/80/1115-3109 $02.00/0

An impurity, N^{10}-methylfolate, associated with methotrexate inhibits folate binding in milk and serum

(*Received* 1 *February* 1980; *accepted* 1 *July* 1980)

High-affinity folate binding proteins are known to be present in milk, serum, leukocytes and various tissues [1]. The biological function of these binders is not fully understood, but they may serve as transport or storage proteins for folates [1]. Of particular interest is the theoretical possibility that at least some of the pharmacological actions elicited by the antileukemic drug methotrexate may be ascribed to interference with the specific protein binding of naturally occurring folate derivatives. However, previous studies concerned with the effect of methotrexate on high-affinity folate binding gave somewhat conflicting results, probably due to differences in the choice of method and concentration of methotrexate. Thus, inhibition of folate binding by methotrexate was reported in milk [2–4], serum [2, 5], leukocyte lysates [6], intestinal and renal brush border membranes [7, 8], whereas other studies showed no inhibition either in hog kidney [9] or in leukocyte lysates [10, 11]. In the latter study [11] methotrexate was purified as described by Gallelli and Yokoyama [12].

The present study deals with the effect of methotrexate,