and confirmed by MAGEE and FARBER[21] for DNA and RNA in rats. Although in vitro alkylation of nucleosides and nucleotides of guanine[18] and deoxynucleotides[19] can also be achieved, the general belief today is that the alkylation in vivo takes place at the level of the macromolecules[16,21-23].

When the patterns of the urinary purines of ^{14}C-MBH-treated and ^{14}C-Na formate-treated mice are compared, the most striking difference is the more than 8 times higher ratio of the specific activity of 7-methylguanine to guanine (24.3) in the ^{14}C-MBH experiment, whereas the ratios of the specific activity of 1-methyladenine to adenine and its metabolic product 1-methylhypoxanthine to hypoxanthine are much lower (7.9 and 4.3, respectively).

These results suggest that the methyl group of MBH not only contributes to the formate pool but is also transferred by by-passing the pool either (and most probably) by a direct transmethylation, or possibly by the route (a) homocysteine → (b) methionine → (c) guanine and adenine; (a) and (c) serve as acceptors and (b) as transmitter of the methyl group.

It is interesting that in a recently published study of the fate of the ^{14}C-labeled methyl group of (methyl-^{14}C-)methionine, MANDEL et al.[24] reported the identification of the same unmethylated and methylated bases in the urine of mice carrying mammary carcinoma as in our studies with P815 leukemic mice. Further studies will be needed to prove or disprove a possible connection between the metabolic pathways of the methyl group of methionine and MBH[25].

Zusammenfassung. Neben der bereits beschriebenen teilweisen Oxydation der endständigen N-Methylgruppe von 1-Methyl-^{14}C-2-*p*-(isopropylcarbamoyl)benzylhydrazin-Hydrochlorid (MBH) (NSC 77213) in vivo[5] lassen die hier aufgeführten Resultate auch auf eine Transmethylierung dieser Methylgruppe schliessen.

W. KREIS, SUSAN B. PIEPHO, and HANNAH V. BERNHARD

Divisions of Experimental and Clinical Chemotherapy, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division of Cornell University Medical College, New York (N.Y., USA), March 22, 1966.

[21] P. N. MAGEE and E. FARBER, Biochem. J. *83*, 114 (1962).
[22] J. L. STARR, Biochim. biophys. Acta *61*, 676 (1962).
[23] M. GOLD, J. HURWITZ, and M. ANDERS, Biochem. biophys. Res. Commun. *11*, 107 (1963).
[24] L. R. MANDEL, P. R. SRINIVASAN, and E. BOREK, Nature *209*, 586 (1966).
[25] Acknowledgments: These studies were supported in part by U.S.P.H.S. Research Contract No. SA-43-ph-2445, Cancer Chemotherapy National Service Center, National Cancer Institute, and U.S.P.H.S. Research Grant No. CA-05826-02, National Cancer Institute; NCI Grant No. CA 08748; by Research Grant No. T 45, American Cancer Society, Delaware Division; and the Campbell Townsend Memorial Grant for Cancer Research from the American Cancer Society. The authors are indebted to Drs. J. H. BURCHENAL, D. A. KARNOFSKY, and I. H. KRAKOFF for their interest in this work; to Dr. A. BENDICH for helpful discussions; and to Dr. W. E. SCOTT of Hoffmann-La Roche Inc., Nutley, N.J., for the supply of unlabeled MBH (Natulan) (Ro 4-6467). We also thank Miss DOROTHY NARY for her skilful technical assistance.

Effect of Menadione on the Phagocytic Activity of Guinea-Pig Polymorphonuclear Leucocytes

Previous work[1] has shown that menadione, an electron acceptor for the oxidation of pyridin nucleotides through the flavoprotein DT-diaphorase[2], strongly increases the oxygen uptake of resting polymorphonuclear leucocytes. The menadione-stimulated respiration is amytal-, rotenone-, antimycin A- and cyanide-insensitive, and it is inhibited by dicoumarol at low concentration. The increased oxidation of $NADPH_2$ by menadione stimulates the oxidation of glucose through the hexosemonophosphate pathway.

The present communication deals with the finding that menadione is a powerful inhibitor of phagocytosis by polymorphonuclear leucocytes and with experiments carried out in an attempt to investigate the mechanism of such inhibition.

Experimental. The experiments on phagocytosis (incubation and cytological examinations) and on metabolic assays were performed as described previously[1,3,4], using guinea-pig polymorphonuclear leucocytes from sterile peritoneal exudate.

Results. (1) Effect of menadione on phagocytosis and its metabolic concomitants in aerobiosis. The addition of menadione $10^{-4}M$, $2 \cdot 10^{-4}M$ to leucocytes incubated in different conditions (Krebs-Ringer phosphate without $CaCl_2$, Krebs-Ringer bicarbonate without $CaCl_2$, tris-buffered NaCl-KCl solution) inhibits phagocytosis of killed opsonized *Staphylococcus aureus* and *Bacillus subtilis*. The extent of inhibition was 80–90% over 30 experiments with different batches of cells. The addition of bacteria fails to stimulate the oxygen uptake and the $C^{14}O_2$ production from glucose-U-C^{14} when menadione is present (Table 1).

Dicoumarol $10^{-5}M$, $10^{-7}M$ slightly increases the respiratory activity of resting cells and has no effect on the extent of phagocytosis. In the presence of dicoumarol, the effect of menadione on the leucocytic respiration is abolished, whereas phagocytosis remains inhibited (Table I).

(2) Effect of menadione on aerobic glycolysis. There are many indications[5-7] on the important energy-supplying role of aerobic glycolysis for phagocytosis in polymorphonuclear leucocytes.

The aerobic production of lactic acid is slightly inhibited by menadione even in the presence of dicoumarol.

[1] F. ROSSI and M. ZATTI, Br. J. exp. Pathol. *45*, 548 (1964).
[2] L. ERNSTER, L. DANIELSON, and M. LJUNGREN, Biochim. biophys. Acta *58*, 171 (1962).
[3] F. ROSSI and M. ZATTI, Experientia *20*, 21 (1964).
[4] F. ROSSI, M. ZATTI, and G. ZOPPI, Experientia *21*, 14 (1965).
[5] H. BECKER, G. MUNDER, and H. FISCHER, Hoppe-Seyler's Z. physiol. Chem., *313*, 266 (1958).
[6] A. J. SBARRA and M. L. KARNOVSKY, J. biol. Chem. *234*, 1355 (1959).
[7] Z. A. COHN and S. I. MORSE, J. exptl. Med. *111*, 667 (1960).

Table I. Effect of menadione on phagocytosis, oxygen uptake and $C^{14}O_2$ production from glucose U-C^{14} by polymorphonuclear leucocytes

	Resting cells				Phagocytosing cells			
	Control	Menadione	Menadione dicoumarol	Dicoumarol	Control	Menadione	Menadione dicoumarol	Dicoumarol
Phagocytosis					Normal	Minimal	Minimal	Normal
Oxygen uptake (μl)	8.2	25.6	14.1	12.3	63.2	26.8	14.9	67.3
$C^{14}O_2$ from glucose U-C^{14} (counts/min)	1426	6234	2468	2180	21685	6326	3017	22121

Each vessel contained about $3 \cdot 10^7$ cells in 2 ml of Krebs-Ringer phosphate (without $CaCl_2$) and 1 μc of glucose U-C^{14} in 5.55 μM (radioactivity $6 \cdot 10^4$ counts/min/μM). When indicated menadione $10^{-4} M$ and dicoumarol $10^{-5} M$ were added. The ratio leucocytes/bacteria (*B. subtilis*) was 1:20. The values are expressed per $3 \cdot 10^7$ cells/30 min of incubation. The phagocytosis was measured by cytological examination on Gram stained films of leucocytes bacteria mixture incubated in the same medium in separate flasks and counting the number of leucocytes containing bacteria. Phagocytosis *normal* or *minimal* means that more than 90% or less than 15% of leucocytes contain bacteria.

Table II. Phagocytosis by polymorphonuclear leucocytes exhibiting a different rate of glycolysis

Conditions of incubation Temperature	Glucose	Menadione	Lactate production	Phagocytosis
38 °C	+	−	1.02	normal
38 °C	+	+	0.75	minimal
38 °C	−	−	0.71	normal
20 °C	−	−	0.29	normal

Each vessel contained about $3 \cdot 10^7$ leucocytes in 2 ml Krebs-Ringer bicarbonate (without $CaCl_2$). When indicated, glucose $5.6 \cdot 10^{-3}$ and menadione 10^{-4} were added. Gas phase 95% O_2 + 5% CO_2. Time of incubation 15 min. Values of lactate production (BARKER and SUMMERSON[10]) are expressed as μM per $3 \cdot 10^7$ cells/15 min. For phagocytosis measurement see Table I.

The significance of this effect has been studied by comparing the phagocytic activity of polymorphonuclear leucocytes exhibiting various rates of aerobic glycolysis, as are obtained by changing the conditions of incubation. The results (Table II) seem to indicate that the inhibition of phagocytosis by menadione is not related to a determinant decrease of glycolysis.

(3) Effect of menadione on the lipid metabolism in aerobiosis. It is known that phagocytosing polymorphonuclear leucocytes exhibit an increased turnover rate of phospholipids[8] and a stimulated incorporation of acetate 1-C^{14} into lipids[9]. Menadione inhibits the incorporation of P^{32} into lipids by 70% and that of acetate 1-C^{14} by more than 90%. The incorporation of P^{32} into acid-soluble phosphorus is inhibited by about 30%. The average figures for the incorporation into lipids of 40 million of resting cells, during 30 min incubation in 2ml tris-buffered medium containing 20 μc P^{32} or 1 μc acetate 1-C^{14}, were 3440 c.p.m. in control cells and 1358 c.p.m. with menadione for the incorporation of P^{32}, and 562 c.p.m. and 55 c.p.m. for the incorporation of acetate 1-C^{14} respectively.

(4) Effect of menadione in anaerobiosis[11]. The addition of menadione inhibits phagocytosis, as well as in aerobic conditions, although it has no effect on glycolysis and on the incorporation of P^{32} into lipids. The incorporation of acetate 1-C^{14} into lipids is still lowered by about 50%.

In some experiments, 20 min were allowed to elapse after the addition of menadione before adding bacteria in order to let the electron acceptor be reduced. Also in this condition phagocytosis is inhibited.

Comment. The results presented here are not conclusive for the interpretation of the mechanism of inhibition of phagocytosis by menadione. Evidence is presented that the effect of menadione is not related to changes in glycolysis, although this pathway is inhibited in aerobic conditions.

Moreover, the fact that dicoumarol fails to remove the inhibition of the phagocytic process while it is effective in abolishing the stimulated respiration, and that menadione inhibits phagocytosis also in anaerobic conditions, seem to indicate that the inhibitory effect of menadione is not related to the electron transport activity for the oxidation of reduced pyridin nucleotides through the flavoenzyme DT-diaphorase. However, it might well be that menadione acts through a mechanism of oxidation, not readily reversible, of sites whose reduced state is needed for the functional activity of the phagocytes.

Riassunto. Menadione in vitro inibisce l'attività fagocitaria di leucociti polinucleati in aerobiosi e in anaerobiosi. Vengono riportate prove sperimentali dalle quali risulta che l'effetto del menadione non è in rapporto con l'inibizione della glicolisi o con l'attività di trasporto di elettroni nella ossidazione dei piridinnucleotidi ridotti tramite una DT diaforasi.

F. ROSSI and G. ZOPPI[12]

Unit Centre 'G. Vernoni' for the Study of Physiopathology, National Research Council and Istituto di Patologia Generale, Università di Padova (Italy),
January 24, 1966.

[8] M. L. KARNOVSKY and D. F. H. WALLACH, J. biol. Chem. *236*, 1895 (1961).
[9] P. ELSBACH, J. exptl. Med. *110*, 969 (1959).
[10] S. B. BARKER and W. H. SUMMERSON, J. biol. Chem. *138*, 535 (1941).
[11] The details of these results will be reported elsewhere.
[12] Present address: Kinderspital, Zürich, Universitätskinderklinik (Switzerland).