analysis of the intensity of ninhydrine rings obtained with amino acid standards of 10–20 μg/ml, referred to as +. At the end of each experiment the sterile conditions of the mixtures were controlled by agar plate tests either for bacteria or for fungi.

Results. Of the catalysts examined, $CuSO_4$ is the most active in producing different amino acids both at 0.05 and 0.1%, especially when nitrogen is supplied by an inorganic source. This synthesis, as well as in the presence of the other catalysts, is characterized by the appearance and disappearance of definite amino acids during the irradiation time with the prevalence of some amino acids. An example of such behaviour is reported in Table I.

The further results with $CuSO_4$, with $FeCl_3$ at 0.05 and 0.1% (also an active catalyst), and with $CoCl_2$ (a good catalyst at 0.05%), are summarized in Table II.

An attempt to potentiate the yield of photochemical synthesis of amino acids has been successful: mixing $CoCl_2$ 0.05% and $FeCl_3$ 0.05% in mixtures where air was the nitrogen source. Here, only glycine and alanine are formed at the beginning and last up to 30 days of light exposure. $NiCO_3$ is practically inactive.

The non-identified ninhydrin rings were presumably due to peptide formation. The tests on the samples kept in the dark showed formation of amino acids detectable in very faint rings with Rf values different from those of photochemically synthesized amino acids.

The mechanism of formation and transformation of amino acids following photocatalysis is not as yet known. Accordingly, it is difficult to understand the prevailing formation of some particular amino acid by the action of specific catalysts. The sterility, controlled carefully throughout the experiments, is a clear indication that formation and transformation of amino acids can occur under abiogenic conditions, as was observed also by MILLER[5] using CH_4 and NH_3 in a cycled system catalysed by electric discharge, and by CULTRERA and FERRARI[6] from glucides and organic acids excited by UV-light. The importance of such a type of photochemical synthesis has been further underscored by recent observations by BAHADUR et al.[7], confirmed by BRIGGS[8], demonstrating that the action of sunlight or of artificial light on sterilized solutions containing mixtures of amino acids and organic catalysts can bring about the formation of units having properties of growth, division and metabolic activity, henceforth called *Jeewanu* (which in Sanskrit means particles of life)[9].

Riassunto. Viene confermato che la sintesi fotochimica degli aminoacidi da paraformaldeide catalizzata da composti inorganici avviene sia in presenza sia in assenza di una sorgente inorganica di azoto fissato (aria). Inoltre viene dimostrato che i tipi di aminoacidi prodotti come la velocità di formazione dipendono dal catalizzatore usato.

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Studies on the Metabolic Fate of the ^{14}C-Labeled Methyl Group of a Methylhydrazine Derivative in P815 Mouse Leukemia

Experimental and clinical evaluation of the cytotoxic agent 1-methyl-2-*p*-(isopropylcarbamoyl)benzylhydrazine hydrochloride (MBH) (NSC 77213) evoked an interest in its reaction mechanism and metabolism. The coincidence of cytotoxic[1], carcinogenic[2], and teratogenic effects[3] revealed a probable attack on the genetic material of the cell, and the breakdown in vivo of DNA by MBH[4] stimulated the search in the same direction. Our interest in this compound was increased by our observation that the terminal N-methyl group was labile[5] and by the considerable interest and speculation as to the biological significance of the normally occurring methylation of RNA and DNA in many species.

The aim of this investigation was to compare the potencies and limits of the formate pool of the mammalian cells in vivo with that of the C_1-unit derived from the N-methyl group of MBH, and to search for methylated and unmethylated purine bases in the urine of P815 leukemic mice treated either with ^{14}C-MBH or ^{14}C-Na formate.

Materials and methods. The two ^{14}C-labeled compounds, MBH (labeled in the terminal N-methyl group) and Na formate, showed a specific activity of 17.0 μc/mg and 74 μc/mg, respectively[6]. Both substances were dissolved in 0.9% saline. 2 series of 30 BDF_1 mice each, ranging in weight from 20–25 g, were inoculated intraperitoneally with 10 million P815 leukemic cells on day 0. On day 6, a group of 10 mice from each series was injected i.p. with 33.33 mg/kg (0.129 mM/kg) of ^{14}C-MBH and 1.47 mg/kg (0.0216 mM/kg) of ^{14}C-Na formate, respectively. The urine of these mice was collected in ice-cooled flasks over a 24 h period. The remaining 20 mice from each series were injected with the same doses of either ^{14}C-MBH or ^{14}C-Na formate on day 7. Their urine was collected 5 h

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following injection of the drugs. The 5 h and 24 h urines of both series were then pooled separately for the extraction studies.

The purine bases were isolated from urine following the procedure described by WEISSMANN et al.[7]. The final separation of the bases was effected using the systems of MARKHAM and SMITH[8] and HOTCHKISS[9]. The spots were extracted from the paper chromatograms with 0.1 *N* HCl, rechromatographed on Whatman paper either in the *n*-butanol-HCOOH-water[8] or a phosphate buffer system[10] and eluted with water. The complete UV-spectrum of these extracts was determined as described by WEISSMANN et al.[7]. An aliquot of the basic solution was added immediately to 10 ml of diatol[11], and the radioactivity was then measured. The specific activities were evaluated as cpm/optical density at the wavelength of the maximum at pH 2.1[12]. The UV-absorption spectra were compared with known samples of guanine, adenine, hypoxanthine 7-methylguanine, 1-methyladenine (compound S of WEISSMANN[7]), and methylhypoxanthine, run in the same paper-chromatography system and extracted and evaluated as above[13].

Results and discussion. As shown in the Table, after i.p. injection of both ^{14}C-MBH and ^{14}C-Na formate, guanine and 7-methylguanine, adenine and 1-methyladenine, hypoxanthine and 1-methylhypoxanthine were found in the 24 h urine (xanthine, but not 7-methylxanthine, was also identified). Several studies indicate that in bacteria[14,15] and Ehrlich ascites cells[16] the methyl group for the methylated trace bases of s-RNA originates primarily from the methyl group of methionine. If this were also true for mammals, we would expect in an experiment, in which ^{14}C-Na formate is used as the sole labeled precursor for purine bases, that methylation of guanine and adenine would not influence the ratios of the specific activity of the methylated to unmethylated bases, i.e. we were to expect a ratio of 1. In the ^{14}C-Na formate experiment, as demonstrated in the Table, the ratios of the specific activity of 1-methyladenine to adenine, and 1-methylhypoxanthine to hypoxanthine (0.99 and 0.94, respectively) come close to the theoretically expected value, whereas the ratio of 7-methylguanine to guanine (2.9) obviously does not. This suggests that ^{14}C-Na formate not only enters the formate pool for use in the de novo synthesis of the C_2 and C_8 positions of the purine bases, but that one part of ^{14}C-Na formate might be transferred either *directly* onto the 7-position of guanine (including a step of reduction) or through the formate pool onto homocysteine, as reported by BERG[17], with subsequent transmethylation of the methyl group of the resulting methionine onto guanine. Although the result of these two, or any other, processes is the same, namely labeled 7-methylguanine, the difference in the specific activities indicates a preference of one of these metabolic pathways or a different size of the pools. A further, but slight, possibility might be a rapid excretion of 7-methylguanine, whereas guanine itself might be re-used for the synthesis of DNA and RNA. However, we would expect a re-use not only of guanine but also of adenine, which is not the case, as the results show. The selective deviation from the expected ratio of the specific activities, as demonstrated here for 7-methylguanine to guanine only, might well be the expression of the more ready accessibility of guanine for methylation or, in general, alkylation. This phenomenon is well known for the alkylation of DNA[18–20] and RNA[20] in vitro with various methylating and alkylating agents and was predicted for in vivo studies by LAWLEY[20]

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Comparison of specific activity of purine bases found in the urine of BDF_1 mice with P815 leukemia after treatment with ^{14}C-MBH and ^{14}C-Na formate

Purine bases	Urine of mice treated with ^{14}C-MBH		Urine of mice treated with ^{14}C-Na formate	
	Specific activity (cpm/optical density)	Ratio of specific activity of methylated to unmethylated base	Specific activity (cpm/optical density)	Ratio of specific activity of methylated to unmethylated base
7-Methylguanine	1505	24.3	1392	2.9
Guanine	62		482	
1-Methyladenine	314	7.9	367	0.99
Adenine	40		370	
1-Methylhypoxanthine	326	4.3	1612	0.94
Hypoxanthine	76		1722	

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.

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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.

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