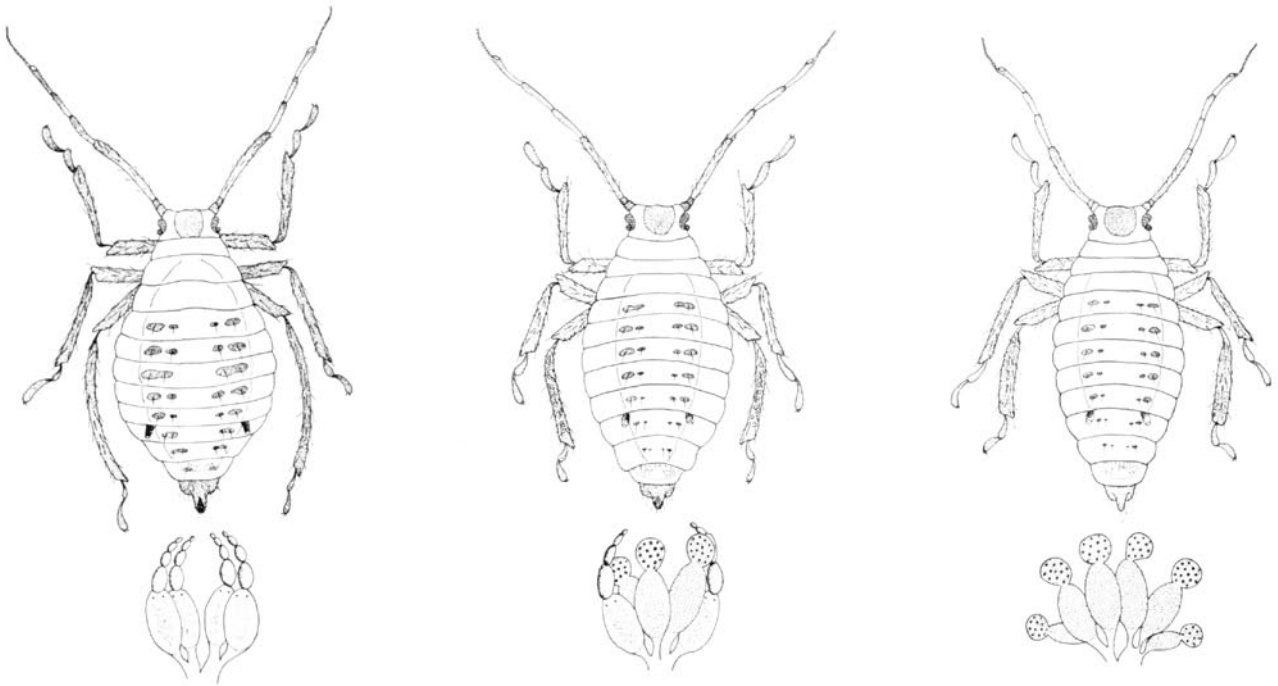

Diagram showing the external features and the ovarian morpholoyg of the three categories of females in the life cycle of *B. brassicae*.

It is therefore possible to regard the ambiphasic females as the result of a perfect 'genic balance' between factors for parthenogenesis and amphigony. Such balance may be obtained during selection experiments when a narrow threshold value is reached which allows the development of both parthenogenetic and amphigonic eggs.

Riassunto. Nel ciclo eterogonico di *B. brassicae* compaiono delle femmine perfettamente intermedie fra le partenogenetiche e le anfigoniche sia per i caratteri morfologici esterni sia per la morfologia degli ovari. Tali femmine compaiono alla fine della selezione in favore delle forme partenogenetiche, che viene effettuata in ambiente regolato per la comparsa delle forme sessuali. Sulla base di queste esperienze, tali femmine, che, per le loro caratteristiche, definisco con il termine di ambifasiche, possono essere considerate come il risultato di un equilibrio dei fattori della partenogenesi e dell'anfigonia, che si raggiunge, attorno ad un valore soglia, nel corso della selezione.

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Istituto di Zoologia, Università di Modena (Italy), December 9, 1964.

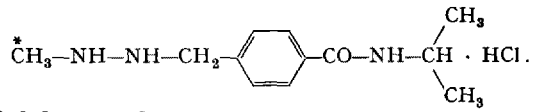
An Antineoplastic C^{14}-Labeled Methylhydrazine Derivative in P815 Mouse Leukemia. A Metabolic Study

A series of methylhydrazine derivatives[1] have been reported to induce chromosomal aberrations in Ehrlich ascites cells[2] and a degradation of DNA in vitro[3]. Accordingly, the effect of these agents in vivo became of interest. Recently, WEITZEL et al.[4] described the formation of formaldehyde as a product of decomposition in vitro of one of these compounds, 1-methyl-2-*p*-(isopropylcarbamoyl)benzylhydrazine hydrochloride (MBH) (Ro 4-6467). They suggested that formaldehyde so formed might act as an alkylating agent. Although formaldehyde in vivo would be expected to contribute to the formaldehyde pool for the de novo synthesis of the purine bases, adenine and guanine (C_2 and C_8 atoms), and the methyl group of thymine, the possibility that it might alkylate any of the purine and pyrimidine bases of DNA and RNA could not be excluded. The fact that a related compound, *p*-hydrazinomethylbenzoic acid, isopropylamide hydrochloride (Ro 6-0233), which differs from Ro 4-6467 by lacking the N-methyl group, is biologically inactive as a tumor inhibitor[5] suggests that the methyl group in MBH

[1] P. ZELLER, H. GUTMANN, B. HEGEDÜS, A. KAISER, A. LANGEMANN, and M. MÜLLER, Exper. *19*, 129 (1963).
[2] A. RUTISHAUSER and W. BOLLAG, Exper. *19*, 131 (1963).
[3] K. BERNEIS, M. KOFLER, W. BOLLAG, A. KAISER, and A. LANGEMANN, Exper. *19*, 132 (1963).
[4] G. WEITZEL, F. SCHNEIDER, and A.-M. FRETZDORF, Exper. *20*, 38 (1964).
[5] W. BOLLAG, personal communication (1964).

plays an important role in the biological reaction mechanism.

We used 1-methyl-C^{14}-2-*p*-(isopropylcarbamoyl)benzylhydrazine hydrochloride (MBH) (Ro 4-6467)[6]



* labeled at this C atom

The aims of the present study were to investigate in vivo the oxidation and release of the methyl group reported by WEITZEL et al.[4], to evaluate its possible role in the metabolism of DNA, RNA and protein of the leukemic cell, and to compare its effect with C^{14}-Na formate[7].

P815 leukemia[8], grown in BDF_1 mice, was used because it was the most sensitive of the three experimental mouse leukemias studied with MBH. The two C^{14}-labeled compounds, MBH and formate, showed a specific activity of 1.74 μc/mg and 17.0 μc/mg, respectively. The two substances were dissolved in 0.9% saline.

Groups of four BDF_1 mice, each weighing 21 to 25 g, were inoculated intraperitoneally with 10 million P815 leukemic cells on day 0. On day 5 or 6 each mouse received a single intraperitoneal dose of 200 mg/kg (0.776 mM/kg) of C^{14}-MBH in one series and 3.075 mg/kg (0.0452 mM/kg) of C^{14}-formate in the second series. After intervals of 15 min, 1 h, 2, 5 or 24 h, the mice were killed by cervical dislocation. The ascites cells were removed by rinsing the opened abdominal cavity with 20 ml of ice-cold 0.9% saline to which 0.8% Na heparinate had been added. The leukemic cells of two mice from each group were collected in a pre-cooled centrifuge tube and the cells freed of ascitic fluid by centrifugation for 5 min at 2000 rpm in an International Centrifuge, Model V, and three subsequent washings with non-heparinized physiological saline. After the cells were frozen overnight, they were extracted following the procedure of Schmidt-Thannhäuser as modified by SCHNEIDER[9]. The radioactivity in the various fractions was determined for the residual proteins by the combustion technique of KALBERER and RUTSCHMANN[10] and by direct addition of 0.2 or 0.4 ml of the liquid extracts to the same amounts of absorption and scintillation mixture used in the combustion method. The uptake of radioactivity was expressed in milli-μ-Mol (mμM) equivalents incorporated into the various ascites fractions of one mouse.

In another experiment, the DNA of ascites cells was extracted by the procedure of KAY, SIMMONS, and DOUNCE[11]. Possible RNA contamination was minimized by incubation with RNA-ase[12] for 1 h at 37°C and dialysis and precipitation with ethanol. After hydrolysis of the DNA with formic acid[13], paper chromatographic analyses were carried out, using the solvent systems of WYATT[14] and HOTCHKISS[15]. The radioactivity of the UV-absorbing spots was measured with the combustion method mentioned above[10].

Figures 1 and 2 show the uptake of radioactivity into the acid-soluble fraction, phospholipids, DNA, RNA, and protein of the leukemic cell after administration of C^{14}-MBH and C^{14}-formate, respectively. The rapid uptake of radioactivity into the acid solubles after C^{14}-MBH (Figure 1) probably indicates (A) a rapid passage of the whole molecule through the cell membrane into the cytoplasm, or (B) a fast oxidation of the labeled methyl group outside (or inside) the cell, and immediate participation of the resulting derivative(s) of that methyl group in the metabolism of the cell, or both. The decrease in radioactivity in the acid-soluble fraction was nearly linear within the 24 h period tested, whereas the radioactivity in the DNA increased almost in inverse proportion to the decrease in the acid-soluble material. RNA, phospholipids and protein showed a rather asymptotic uptake. When these results are compared with the uptake of radioactivity after injection with C^{14}-formate (Figure 2), the most striking differences are a much lower incorporation of activity in the acid-soluble fraction and a much more rapid uptake in the phospholipids, RNA, DNA, and protein.

Although these experiments indicate that all or part of the MBH molecule is incorporated into DNA, RNA, and protein, studies were extended to determine first which part of the molecule is taken up into DNA.

DNA was isolated from ascites cells of P815 leukemic mice which had been treated either with C^{14}-MBH or C^{14}-formate, as described above. The DNA was hydrolyzed and chromatographed on Whatman No. 1 filter paper. The resulting UV-absorbing spots were partly extracted and partly combusted. Using the paper chromatography system of WYATT[14], the results (Table) show the radioactivity and base composition.

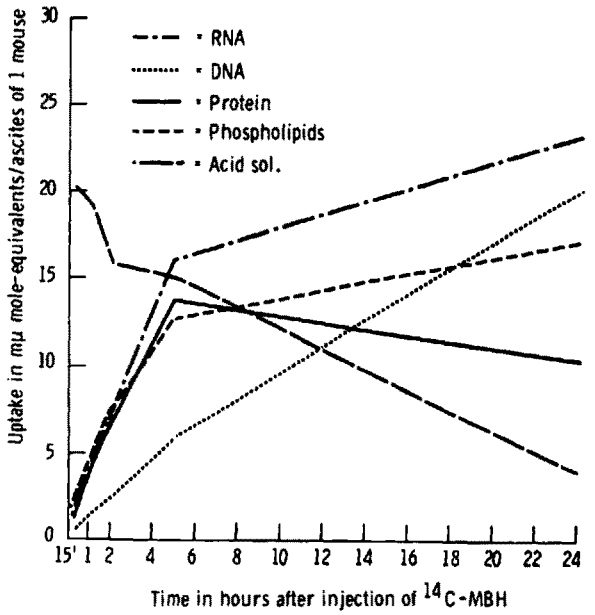


Fig. 1. Uptake of radioactivity into 5 fractions of P 815 leukemic cells after i.p. injection of ^{14}C-MBH.

[6] Kindly supplied by F. Hoffmann-La Roche AG, Basel (Switzerland), through the courtesy of Dr. W. BOLLAG.

[7] Purchased from the New England Nuclear Corporation, Boston (Mass.).

[8] T. B. DUNN and M. POTTER, J. Nat. Cancer Inst. *18*, 587 (1957).

[9] W. C. SCHNEIDER, J. biol. Chem. *164*, 747 (1946).

[10] F. KALBERER and J. RUTSCHMANN, Helv. chim. Acta *44*, 1956 (1961).

[11] E. R. M. KAY, N. S. SIMMONS, and A. L. DOUNCE, J. Am. chem. Soc. *74*, 1724 (1952).

[12] Purchased from C. F. Böhringer & Söhne GmbH, Mannheim (Germany), through California Corporation for Biochemical Research, Los Angeles (Calif.).

[13] A. BENDICH, *Methods of Enzymology* (Ed.: CHARGAFF and DAVIDSON; Academic Press, New York 1957), vol. 3, p. 715.

[14] G. R. WYATT, Biochem. J. *48*, 584 (1951).

[15] R. D. HOTCHKISS, J. biol. Chem. *175*, 315 (1948).

Rf values and UV-spectra of the extracted spots found with the second system employed, that of HOTCHKISS[15], were also identical to those of the original, commercially available adenine, guanine, cytosine and thymine, respectively. Radioactivity could be found only in adenine, guanine and thymine in cells treated with C^{14}-MBH and C^{14}-formate, respectively. Furthermore, the spots of these bases were the only ones over the whole length of the paper chromatogram where a distinct radioactivity above background was found. Although the distribution of counts per min (cpm) per spot in the MBH experiment did not parallel those in the formate experiment, the patterns of distribution of radioactivity were similar in both experiments. These results indicate that in vivo the methyl group of MBH is metabolically labile, since it was oxidized and incorporated into adenine, guanine and the methyl group of thymine. Studies are under way to investigate whether methylation of the purine or pyrimidine bases also occurred[16].

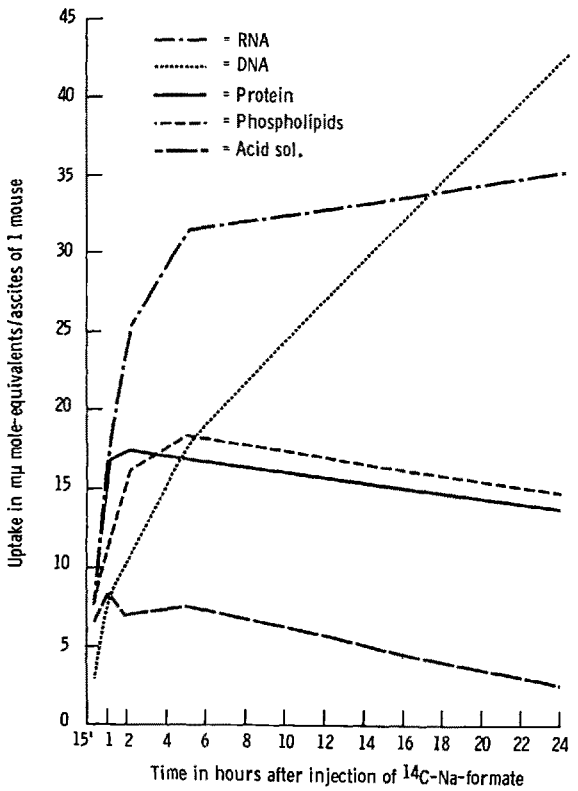


Fig. 2. Uptake of radioactivity into 5 fractions of P 815 leukemic cells after i.p. injection of ^{14}C-Na formate.

Comparison of deoxyribonucleic acids extracted from ascites of P815 leukemic cells treated with C^{14}-MBH and C^{14}-formate

Base	Treated with C^{14}-MBH		Treated with C^{14}-formate	
	cpm[a]	Molar ratio[b]	cpm[a]	Molar ratio[b]
Guanine	72	19.6	796	19.8
Adenine	108	29.5	776	28.9
Cytosine	0	21.0	10	21.0
Thymine	46	29.9	536	30.8

[a] cpm/spot (mean value of 3 to 4 determinations). [b] Mean value of 4 to 8 determinations.

Zusammenfassung. Die endständige N-Methyl-Gruppe von 1-Methyl-C^{14}-2-*p*-(isopropylcarbamoyl)benzylhydrazin-Hydrochlorid (MBH) (Ro 4-6467) erwies sich in In-vivo-Experimenten metabolisch labil, wird teilweise oxydiert und Bestandteil des Formiatpools.

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Divisions of Experimental and Clinical Chemotherapy, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division of Cornell University Medical College, New York (USA), December 9, 1964.

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Effect of Na^+, Ca^{++} and Mg^{++} on the O_2 Consumption of the External Medulla of Dog Kidney

The effect of the external Na^+ concentration on the O_2 consumption of the external medulla of dog kidney slices was investigated by ULLRICH and PEHLING[1]. They demonstrated, by means of a particular technique, that a correlation exists between these two variables. No information is apparently available, however, on the influence exerted by the external depletion of Ca^{++} and Mg^{++} on the O_2 consumption associated with the Na^+ transport. This work was designed with the purpose of finding out this influence and incidentally establishing the functional relationship between Na^+ concentration and O_2 consumption.

The dogs were killed by a shot in the head, the kidneys were removed immediately, placed on iced glass and the external medulla was cut with a razor blade. The slices were incubated in Krebs-Ringer buffered with Tris containing also glucose and α-ketoglutarate.

[1] K. J. ULLRICH and G. PEHLING, Pflügers Archiv. ges. Physiol. *267*, 207 (1958).