were visible after 8 h of incubation. All these rosettes consisted of a mononuclear cell surrounded by various numbers of myeloblasts (Figures 2, 3, 5 and 6). The central cells had usually an oval-, kidney- or horseshoe-shaped nucleus with one or several small nucleoli. The abundant, pale and poorly defined cytoplasm appeared homogenous. By cytological criteria, these cells were classified as monocytes. Frequently, they were found to contain a phagocytozed cell or cell remnants (Figures 5 and 6). Leukemic blast cells with a large, round or oval nucleus containing one or several prominent and deeply stained nucleoli were assembled around the monocyte. These blast cells were usually elongated and oriented towards the central cell, the cytoplasmic process often in direct contact with the surface of the monocyte (Figure 5).

Additional experiments indicated that the initial cell concentration in the culture medium was an important factor in obtaining well-defined rosettes. If the concentration exceeded 3×10^6 cells/ml, a loose monolayer developed and the myeloblasts tended to cluster irregularly around the monocytes (Figure 4).

No rosettes were evident in multiple white blood cell cultures from the 3 patients with AML, whose peripheral blood *lacked* monocytes (Table, group D; individuals Nos. 6–8).

Discussion. In this study, where leukocyte cultures from patients with AML, CML, CLL and cultures from healthy controls were checked for the presence of rosettes, such cellular arrangements were, without any exception, observed only in leukocyte cultures from AML-patients *having monocytes* in the peripheral blood. The presence of phagocytozed material in many of the centrally located cells in the rosettes indicates that at least part of these cells had, in the cultures, transformed into macrophages. Such a transformation of monocytes in vitro is known to occur within a few hours of incubation[6].

Although the significance of the spontaneous formation of rosettes in these AML-leukocyte cultures is, at the present time, far from being clear, one might speculate that mechanisms similar to those operative in other rosette-forming systems[1–3] may play a role. Thus, a rosette-like arrangement of erythrocytes around macrophages could be observed, when normal sheep red blood cells were added to lung macrophages from guinea-pigs which had been actively immunized with sheep erythrocytes[2]. The characteristic binding of these 2 cell types was thought to be due to the presence of *cytophilic antibodies*[7,8] elaborated in the animal, which conferred upon its macrophages the ability to adsorb the specific antigen.

Should a similar mechanism be responsible for the rosette-formation in the AML-leukocyte cultures, this would indicate, in the patients' serum, the presence of specific antibodies against the leukemic cells. The finding that gammaglobulin G or its Fc-fragment inhibits, in solution, the formation of rosettes[4], could thereby explain the absence of such cellular arrangements in vivo, i.e. in the AML-patients' peripheral blood[9].

Total white blood cell counts[a], percentage of monocytes in the conventional dry smears and occurrence of rosettes in the leukocyte cultures on Millipore filters

Group	Patient No.	Total cell count (cells/mm³)	% of monocytes (dry smears)	Rosettes in culture
A	1	5,200	5	—
Healthy	2	7,100	3.5	—
controls	3	3,500	4	—
	4	7,200	4	—
	5	4,550	8	—
B	1	106,600	0	—
Chronic	2	111,000	0	—
lymphocytic	3	298,000	0	—
leukemia CLL				
C	1	74,000	1.5	—
Chronic	2	49,000	3	—
myelocytic leukemia CML				
D	1	12,800	4	++
Acute	2	5,200	2	++
myeloblastic	3	186,000	1.5	++
leukemia	4	216,000	2	++
AML	5	23,000	22.5	++
	6	49,000	0	—
	7	168,000	0	—
	8	37,500	0	—

[a] Groups B and C: values before resumption of therapy. Group D: values before initiation of therapy.

Zusammenfassung. In Monozyten enthaltenden Leukozyten-Kulturen von Patienten mit akuter myeloischer Leukämie entstehen nach ungefähr 8 h Inkubationszeit Rosetten, die aus einem von Myeloblasten umgebenen Monozyten bestehen. In Analogie zu anderen in vitro-Systemen, in welchen sich eine Rosettenbildung beobachten lässt, könnte diese zelluläre Reaktion auf dem Vorhandensein von spezifischen Antikörpern im Patienten-Serum beruhen, welche gegen die leukämischen Myeloblasten gerichtet sind.

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[9] Acknowledgments. This work has been supported by the Julius Müller Foundation for Cancer Research, Zürich, and by Grant No. 4804 from the Swiss National Foundation for the Advancement of Scientific Research. We thank Miss M. SCHÄR, Mrs. J. STRASSER, Mrs. A. HAUSWIRTH and Mr. W. F. SCHERLE for excellent technical assistance.

Relation between Carcinogenicity and Metabolic Reduction of 4-Nitroquinoline 1-Oxide Derivatives

The reduction product of 4-nitroquinoline-1-oxide (4NQO), 4-hydroxylaminoquinoline-1-oxide (4HAQO) has been reported to be carcinogenic[1–3]. The metabolic pathway which converts 4NQO to 4HAQO was detected in animal tissues[4–6]. This report compares the susceptibility of a number of derivatives of 4NQO to be metabolized to the hydroxylamino compounds in relation to their carcinogenicity, as part of studies on their mechanism of action.

Materials and methods. All compounds were synthesized in this Institute. Compounds dissolved in a small amount

of propylene glycol were added to the reaction mixture at a concentration of $3 \times 10^{-4} M$. Reaction mixture consisted of $0.05 M$ phosphate buffer (pH 7.0), 0.07% bovine serum albumin, $1 \times 10^{-4} M$ $NADH_2$ and an appropriate amount of enzyme preparation, which was partially purified with ammonium sulfate fractionation from 105,000 *g* supernatant of rat liver as described before[5]. The reaction was started by the addition of $NADH_2$ and the change in the optical density at 340 nm was followed at 30 °C with a Hitachi recording spectrophotometer. In this system it was proved that 4NQO was converted to 4HAQO at the expense of oxidation of $NADH_2$, that is 4NQO served as a hydrogen acceptor and $NADH_2$ served as a hydrogen donor, and 4HAQO was not reduced further to 4-aminoquinoline-1-oxide[5].

The reduction rates of the nitro compounds were expressed as the initial rates of $NADH_2$ oxidation/mg protein/min. The relative initial rates for these derivatives were calculated with reference to the rate of constant for 4NQO.

Results and discussion. The data are summarized in the Table. All derivatives of 4NQO with substitutions at positions 2, 6 or 8 were active as hydrogen acceptor, 6,7-dichloro-4NQO being the most active. All these are carcinogenic[7-11]. Two analogs of 4NQO, 3-nitroquinoline-1-oxide and 5-nitroquinoline-1-oxide, did not serve as hydrogen acceptors in this system, and were not carcinogenic[8]. The Table also lists the polarographic reduction potential ($-E_{1/2}$) of the nitro group to hydroxylamino group in solution at pH 6.98[8,12,13]. Some correlations can be noted between 2 kinds of reduction processes, as expected. Thus, those which have $|E^1/_2|$ values of more than 0.20 V were all inactive in the enzymatic reduction under the conditions described.

In conclusion, 4NQO derivatives which could not be converted enzymatically to 4HAQO derivatives are not carcinogenic. The metabolic pathway of 4NQO derivatives to their 4HAQO derivatives appears to be an essential step for carcinogenesis. We have previously reported that 4NQO formed a covalently-bound compound with DNA after in vivo injection, but 3-methyl-4NQO failed to do so[14]. Metabolic conversion to hydroxylaminoderivatives and modification of DNA are apparently related to the development of carcinogenic potency of 4NQO derivatives.

All derivatives of 4-nitroquinoline-1-oxide with substitutions at positions 2, 6 or 8 which could be enzymatically reduced to corresponding derivatives of 4-hydroxylaminoquinoline-1-oxide were carcinogenic. Derivatives with substitution at position 3 were not enzymatically reduced and non-carcinogenic[15].

Zusammenfassung. Untersuchungen über eventuelle Zusammenhänge zwischen der Karzinogenität gewisser Nitrochinolinderivate und deren Fähigkeit, enzymatisch zu Hydroxylaminverbindungen reduziert zu werden.

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Relation between enzymatic reduction, reduction potential and carcinogenicity of 4NQO derivatives

Compound	Relative rate of reduction	Reduction potential[a] $-E_{1/2}$	Carcinogenicity[b]
4-nitroquinoline-1-oxide	1.00	0.174	+ (11)
2-methyl-4NQO[c]	0.22	0.197	+ (10)
6-nitro-4NQO	3.09	0.154	+ (7)
6-chloro-4NQO	4.15 (5)	0.157	+ (10)
8-methyl-4NQO	0.54	0.187	+ (8)
6,7-dichloro-4NQO	5.60	0.144	+ (8)
3-methyl-4NQO	0.02	0.258	— (7)
3-methoxy-4NQO	0.02	0.270	— (8)
3-nitroquinoline-1-oxide	0.01	0.245	— (8)
5-nitroquinoline-1-oxide	0.00	0.260	— (8)
4-nitroquinoline	0.00	0.218	— (10)

[a] See references [8,12,13]. [b] Figures in parentheses are reference numbers. [c] 4NQO is 4-nitroquinoline-1-oxide.

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[15] Authors are grateful to Dr. Y. Kawazoe of this Institute for generous gifts of 4NQO derivatives and critical discussions. They wish to express their thanks to Dr. J. H. Weisburger, National Cancer Institute, Bethesda, Md., for reading this manuscript.

Primary Antibody Response in Mice Bearing Leukemia L1210[1]

Immunosuppression in mice infected with murine leukemia viruses has been demonstrated by several investigators[2-5]. However, contradictory results have been obtained with transplanted tumors. Thus, impaired antibody production in animals bearing carcinomas and lymphomas has been reported[6-8], while almost normal immunological response has been found by others in mice bearing Ehrlich or mammary carcinomas[9,10]. Little is known about the immunological reactivity of mice bearing leukemia L1210, with the exception of a few reports[6,8] which seem to indicate that the tumor induces a slight depression of heterohemolysin, but not hemagglutinin, production.

Because of the wide use of leukemia L1210 as a tool in chemotherapy studies, and the contributory role played by the host's immunological response to the efficacy of