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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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

Antibody plaque-forming cells in spleen of leukemic (L1210) and nonleukemic mice. Plaque-forming cells per spleen ($\times 10^3$)

Groups	Days after SRBC immunization Day 2	Day 4	Day 6	MST (days)
C	11.1 (10.1–12.2) [22]	280.8 (269.8–292.3) [18]	45.2 (35.7– 57.2) [6]	
L_2	12.4 (11.3–13.6) [5]	371.4 (331.9–415.5) [5][a]	168.1 (150.7–187.4) [3][b]	8
L_3	9.7 (9.0–10.5) [9]	452.7 (435.3–470.8) [11][b]	157.2 (131.1–175.9) [4][b]	8
L_4	10.5 (8.9–12.6) [9]	167.9 (150.1–187.9) [8][b]		8.5
L_5	13.6 (11.6–15.9) [12]	127.6 (101.5–160.6) [3][b]		8.5

Geometric means of PFC/spleen (in parentheses the values of the mean minus and plus one standard error calculated after logarithmic transformation; in brackets the number of animals used for each determination). Values were obtained in normal and leukemic mice after single i.v. injection of 4×10^8 sheep erythrocytes (SRBC). Background values for nonimmunized mice were 140 (90.5–185) PFC/spleen in nonleukemic CDF_1 male mice and 210 (152–261.5) PFC/spleen in leukemic mice, 8 days after transplantation of 10^5 L1210 cells i.p. ($P > 0.1$). C, nonleukemic controls; L, leukemic mice, transplanted i.p. with 10^5 L1210 ascites cells and immunized with SRBC 2 (L_2), 3 (L_3), 4 (L_4) and 5 (L_5) days after tumor transplantation; MST, median survival time of 8 mice. [a] $P < 0.05$. [b] $P < 0.01$.

antitumor drugs[11–13], we directed our attention to the kinetics of the immune response at several different stages of leukemic growth in mice. The present communication concerns the study of the primary humoral antibody response employing the direct plaque-forming cell (PFC) method in leukemic mice (L1210) immunized with sheep erythrocytes (SRBC) 2, 3, 4, or 5 days after tumor transplantation.

CDF_1 male mice (BALB/c female × DBA/2 male)F_1, weighing 22–26 g were randomized and transplanted i.p. with 10^5 L1210 leukemic cells on days 0, 1, 2 or 3. On day 5 all leukemic and nonleukemic control mice were injected i.v. with a single dose (4×10^8) of washed SRBC suspended in 0.5 ml of saline. Mice were sacrificed on days 2, 4, and 6 after immunization and the PFC per spleen were determined employing the method of JERNE et al.[14], with some modifications in the preparation of spleen cell suspensions as suggested by SHEARER et al.[15]. In addition, adequate numbers of leukemic mice were included in all groups so that the survival time could be determined.

The results of the experiment are summarized in the Table. The data show that the PFC response is higher on days 4 and 6 after injection of SRBC in leukemic mice immunized 2 or 3 days after tumor transplantation (groups L_2, L_3 in the Table), than in nonleukemic mice. (It seems noteworthy that even a spleen of group L_3, collected 15–30 min after leukemic death, showed a higher PFC value than the controls). Leukemic mice, however, immunized 4 or 5 days after tumor transplantation (groups L_4, L_5), showed impaired PFC response on day 4 after SRBC injection. Thus, the influence of leukemic development on the primary antibody response was such that there was a critical change between days 3 and 4 after tumor transplantation. Injections of SRBC at the various times relative to leukemic inoculation, had no influence on the survival time of the leukemic mice.

These findings indicate that leukemic growth, including accumulation of ascites and infiltration of spleen with leukemic cells and tumor-induced cachexia on days 4 and 6 after SRBC, did not depress the antibody response in animals immunized within 3 days after tumor transplantation. This is in agreement with the findings of GELZER and DIETRICH[10] with Ehrlich carcinoma. Tumor-bearing mice evidently are able to support the proliferation of tumor cells, as well as of immunocompetent cells, and no interaction is expected to occur even if the 2 distinct processes take place in the same organ (spleen). However, it is difficult to explain the increased PFC per spleen in leukemic mice. A similar observation was made with a murine leukemia virus by SIEGEL et al.[5]. They showed a higher number of PFC per spleen in animals immunized with SRBC along with Rauscher virus infection. No explanation for the phenomenon was given, although an increase in phagocytic index, which accompanies some antitumor immune reactions[16], could be implicated in our observation of the more pronounced immunological response. On the other hand, the reduction of PFC per spleen in mice immunized with SRBC 4 or 5 days after leukemic transplantation could be attributable to other factors that might play a predominant role in more advanced stages of the disease. It has been shown that L1210 is antigenic for CDF_1 mice[17]. Also, it has been calculated that more than 50×10^6 tumor cells could be found in ascitic fluid and blood in a mouse 4 days after i.p. transplantation of 10^5 L1210 cells[18]. This suggests that an antigenic competition could account for at least a part of the observed immunosuppression. Also, the parallel findings for the immune response of L1210 leukemic mice and Rauscher virus infected mice[5] would suggest the possibility of a concomitant virus infection, even

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though attempts to isolate an L1210-associated virus have been unsuccessful[19].

In conclusion, the PFC kinetic pattern in leukemic mice indicates that growth of L1210 leukemia does not depress the primary humoral antibody response of the host, unless the antigenic stimulus is given at a later stage of tumor development. In such case moderate immunosuppression was observed at 4 days after administration of the antigen.

Riassunto. La risposta immunitaria primaria a globuli rossi di montone, studiata mediante la tecnica delle placche di Jerne in topi CDF_1 normali o portatori di leucemia L1210, indica che lo sviluppo neoplastico deprime la formazione di anticorpi umorali solo nel caso in cui l'antigene venga iniettato in fase avanzata della malattia.

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Androgens and Erythropoiesis in Bone Marrow. II. Effect of Testosterone Propionate on ^{59}Fe Concentration in Erythrocytes and Bone Marrow

The erythroid maturation of bone marrow in young, adult, Lewis male rats revealed a shift from polychromatic and orthochromatic normoblasts to basophilic normoblasts beginning 4 weeks after gonadectomy. Such a distribution of normoblasts returned to normal after the rats were injected s.c. with testosterone propionate daily for 7 days[1].

The transit time (maturation time) of polychromatic and orthochromatic normoblasts in bone marrow of normal rats was estimated to be 22 h while the transit time of pronormoblasts, basophilic normoblasts, polychromatic and orthochromatic normoblasts accounted for a total of 73 h[2]. The rate of division of erythroid stem cells and/or their precursors, leading to differentiation of pronormoblasts, was accelerated by erythropoietin (ESF) in 5-FU treated mice and the first ESF-dependent erythrocytes appeared in the peripheral blood 3 days following ESF administration[3]. Erythropoietin production increases following testosterone administration and this is considered, at least in part, the determining factor for the erythropoietic activity of androgens[4].

In view of these premises we investigated the time of the earliest erythropoietic effect induced by administration of testosterone propionate to young, adult Lewis male rats gonadectomized 4 weeks before. The hormone was injected s.c. at the dose levels and regimens indicated in tables. The total volume of each injection was 0.5 ml based on SSV (mixture of isotonic saline, polysorbate 80, carboxymethylcellulose and benzyl alcohol). The erythropoietic activity was expressed as percent of ^{59}Fe uptake by erythrocyte and bone marrow following intracardial administration of 1 μc of ^{59}Fe in 0.5 ml saline. The ^{59}Fe was administered with the hormone, when it was given in a single administration, and with the last injection of testosterone propionate, when the animals were treated daily for 7 days.

The animals were sacrificed by etherization immediately after the blood was taken and the bone marrow was blown out, with a 5 ml syringe, from the right femur with a No. 18 gauge needle. The marrow was then weighed and treated for measurement of radioisotope[5]; radioactivity was counted in a well-type liquid scintillator counter. An 0.1 ml aliquot of red cells washed 3 times with saline was used for measuring ^{59}Fe uptake by the same method employed for the bone marrow determination. The ^{59}Fe uptake has been reported as percent of total dose injected into whole circulating erythrocyte (2.37 ml erythrocyte/100 g body wt.) and per 100 mg of bone marrow.

Each experiment was duplicated. The results were reported as means $\pm$ S.D. and the Student's test was used for determining p value. Any change showing $p < 0.01$ was considered significant.

Measuring ^{59}Fe uptake 24 h following the radioiron administration (Table I) revealed an enhancement of erythropoietic activity due to 0.4 mg of testosterone propionate injected daily for 7 consecutive days. The ^{59}Fe content decreased in bone marrow and increased in erythrocytes. When radioactivity was measured at 48 and 72 h after ^{59}Fe administration any changes that might have occurred were apparently overcome by the on-going normal rate of erythroid maturation. The 24 h change of ^{59}Fe content in erythrocytes and bone marrow characterized the erythropoietic effect of testosterone propionate in animals with erythropoiesis impaired only by large depletion of endogenous androgens (and not by any other artifact such as polycythemia or starvation). The results could be revealing the cumulative effect of repeated doses of testosterone propionate rather than an immediate effect of the steroid.

The injection of different amounts of testosterone propionate given in single doses to gonadectomized animals showed that a s.c. injection of 1.6 mg of the androgen simultaneously with 1 μc of ^{59}Fe injected intracardially still depletes radioiron from bone marrow and increases its content in erythrocytes within 24 h (Table II). In additional trials bleedings prior to 24 h were tested but no significant changes of ^{59}Fe incorporation were noticed.

These findings are in agreement with the morphological changes previously observed in bone marrow[1]. In fact, since the transit time of polychromatic and ortho-

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