Totally 12 male C_3H mice (b.wt. 25 g) were irradiated (Co^{60}-850R) and each was injected i.v. with 5×10^4 bone marrow cells. 6 of the animals were then injected i.v. with 4 μCi ^{14}C-nicotine on the 7th day after irradiation and another 6 mice received ^{14}C-nicotine on the 10th day after irradiation. The survival times were 30 min and 4 h (3 animals at each time interval) for both groups. After the killing, either the whole mouse or the removed spleen were embedded in carboxymethylcellulose, sectioned and subjected to whole-body autoradiography as described above. Several irradiated mice which were injected with ^{14}C-nicotine (but not with bone marrow cells) were run as controls to ensure that the irradiation itself did not result in changes in the distribution pattern of ^{14}C-nicotine.

To differentiate between the erythropoietic and granulopoietic colonies, serial sections (10 μm thick) of the spleens were made from mice with bone marrow transplanted and subsequently injected with ^{14}C-nicotine. One section was always exposed on film (autoradiographed) and stained afterwards with hematoxylin-eosine. The adjacent section was then subjected to a cytochemical method for demonstration of peroxidase which is specific for neutrophilic and eosinophilic granulocytes and its precursors[11,12]. In this way it was possible to compare directly the uptake of label in the peroxidase positive- (granulocytic) and the peroxidase-negative (erythropoetic) colonies.

The whole-body autoradiograms of normal mice injected with ^{14}C-nicotine (Figure 1) showed a marked accumulation of radioactivity in some organs of the lymfomyeloid system, such as the bone marrow and the spleen (mainly red pulp). The accumulation of radioactivity was highest 5 min after injection, exceeding that of the blood by about 4 times. After 30 min the labelling intensity dropped to about half the original level, but remained unchanged up to 8 h following the injection of ^{14}C-nicotine. At that time no or very little radioactivity could be seen in the blood and most of the tissues (Figure 1). At shorter time intervals, the uptake of radioactivity could also be observed in the thymus (cortex) and lymph nodes but it soon disappeared. After 4 h no radioactivity was detectable in these tissues. The irradiation of the mice did not seem to result in any change of the nicotine distribution picture excepting the hemopoietic tissues (in which the lack of radioactivity coincides with the depletion of highly radiosensitive hemopoietic cells).

On the autoradiograms of irradiated mice grafted with the bone marrow cells, a high and prolonged accumulation of radioactivity could be seen in the splenic colonies at all time intervals after the injection of ^{14}C-nicotine (Figures 2 and 3). The intensity of labelling in the colonies was about 4 times higher than in the rest of the splenic tissue and remained on that level during the entire period of the investigation (Figure 3). Actually the uptake of label was of the same order of magnitude as the labelling in the other organs of the body showing a pronounced accumulation of nicotine – such as the bronchi, the liver and the excretory pathways (Figure 2).

Among the colonies, the larger ones, detectable mainly on the 10th day after irradiation, seemed to exert the highest uptake. Since this sort of colony is mostly of the erythrocytic type[13], the result would indicate an affinity of nicotine and/or its metabolities for the erythropoietic cell precursors. This was further confirmed when the uptake of radiactivity was compared with the peroxidase activity (specific for the granulocytopoetic cells – see above) in the colonies. In the small colonies that exerted a positive reaction on peroxidase, a very slight uptake of radioactivity was seen as compared with the large peroxidase-negative ones (see Figure 3). Thus the precursor cells able to take up and accumulate radioactivity after the injection of ^{14}C-nicotine seem to be of the erythropoietic type.

No attempts were made in the present investigation to determine whether the radioactivity in the bone marrow and/or in the splenic colonies represents the nicotine or its metabolites. However, the experiments with various tissues in vitro have shown the liver to be the only organ with a significant capacity to metabolise nicotine[14]. This fact, together with the early localization of nicotine in the bone marrow observed in this investigation, would suggest that at least part of the radioactivity accumulated in the hemopoietic tissue represents the unchanged drug[15].

The present results do not allow any conclusions on the functional significance of nicotine in the hemopoietic tissues. However, the specific and prolonged accumulation of the drug in the erythropoietic cells could indicate some kind of nicotine interference with the proliferative capacity and/or maturation of the erythrocyte precursors – as well as an interference with the functional activity of the mature erythrocytes. Further experimental studies are needed to elucidate this point, and also to explain the precise functional significance of nicotine in the hematopoietic tissues.

Zusammenfassung. Nach intravenöser Injektion von ^{14}C-Nikotin wurde bei der normalen Maus mit Hilfe der Ganzkörperautoradiographie im Knochenmark und in der roten Pulpa der Milz eine kräftige und langanhaltende Ansammlung der Radioaktivität festgestellt. Kombinierte autoradiographische und histochemische Untersuchungen der Milz bestrahlter, mit Knochenmark transplantierter Mäuse haben weiter gezeigt, dass das radioaktive Nikotin und/oder dessen Metabolite innerhalb des hämatopoietischen Gewebes hauptsächlich in den erythropoietischen Zellen lokalisiert ist.

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Genetic Influence on Development of Reticulum Cell Sarcomas in SJL/J Mice

Initially, it was reported that SJL/J mice exhibit a distinct elevation in the plasma levels of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) after infection with the LDH virus[1]. It was then shown that this unique response is a recessive trait under the control of an autosomal gene[2]. This paper presents initial evidence for

a role of the gene in the susceptibility of SJL/J mice to development, in high incidence, of Hodgkin's-like reticulum cell sarcomas[3].

Materials and methods. Animals. SJL/J and BALB/c mice, 6 to 8 weeks old, were crossed reciprocally. These matings, as well as those to obtain F_2 generation animals, were established on the basis of 2 females and 1 male per cage. Only female offspring, weaned at 5 weeks of age, were used in the experiments because of excessive fighting among male cagemates.

Genotype determination. All animals, with the exception of parental mice, were infected at 6 to 8 weeks of age by i.p. injection of 0.1 ml of pooled mouse plasma, diluted 10^{-1} in cold Hank's balanced salt solution, containing $10^{7.5}$ ID_{50}/ml of LDH virus[4]. Plasma samples for enzyme assays were obtained at 96 h postinoculation as described previously[1]. LDH and MDH activities were determined by the methods of HENRY et al.[5] and MEHLER et al.[6], respectively. One unit of enzyme activity is defined as the amount of enzyme which produces a decrease in absorbancy of 0.001/min at 340 nm resulting from the oxidation of NADH to NAD^+. All tests were made at 25 °C in 3 ml reaction mixtures; the results are expressed in units of enzyme activity per ml of plasma.

Mice homozygous for the gene in question were identified in the F_2 generation by 'exaggerated' plasma enzyme levels (LDH and MDH activities like those of the SJL/J strain). F_1 and F_2 heterozygotes, as well as homozygotes in the F_2 generation, had 'elevated' plasma LDH and MDH levels like those of the BALB/c strain[2]. The possibility that the results might be altered by the method of genotype determination was discounted since LDH virus infection has been shown to have no effect on the incidence of the SJL/J neoplasm[7].

Table I. Incidence of reticulum cell sarcomas in female F_1 and F_2 generation mice produced by reciprocal crosses between strains SJL/J and BALB/c

Generation	Plasma enzymes (U/ml) [a]		No tumors	
	LDH	MDH	no mice [b]	%
P (SJL/J)	13,250 ± 792	3,150 ± 197	7/10	70.0
(BALB/c)	5,000 ± 330	1,550 ± 148	0/10	0.0
F_1	4,850 ± 157	1,400 ± 64	3/33	9.1
F_2	4,450 ± 123	1,500 ± 57	3/40	7.5
	11,900 ± 577	3,000 ± 111	5/21	23.8

[a] Mean ± S.E. [b] 52 weeks of age.

Table II. Incidence of reticulum cell sarcomas in cyclophosphamide-treated, female F_2 hybrids of strains SJL/J and BALB/c

Plasma enzymes (U/ml) [a]		No tumors	
LDH	MDH	no mice [b, c]	%
5,500 ± 116	1,650 ± 60	3/20	15.0
13,350 ± 801	4,000 ± 201	5/11	45.5

[a] Mean ± S.E. [b] 52 weeks of age. [c] Data from mice receiving i.p. injections of cyclophosphamide (75 mg/kg) for 6 and 10 weeks were similar and thus combined.

Immunosuppression. Beginning 2 weeks after infection with the LDH virus, 17 female F_2 mice received 6 weekly injections of an immunosuppressive agent, cyclophosphamide (Cytoxan). The drug was prepared at a concentration of 15 mg/ml in cold 0.85% saline and administered i.p. (75 mg/kg). In a second group, 16 F_2 animals were treated similarly for 10 weeks.

Neoplasms. Mice were killed and necropsied at 52 weeks of age. Gross observations of enlargement of the spleen, Peyer's patches, mesenteric lymph node complex and other lymph nodes were accepted as evidence of reticulum cell sarcoma. In those cases in which the gross evidence seemed equivocal, sections of appropriate organs were examined histologically before a diagnosis was made.

Results and discussion. Table I shows that 9.1% of the F_1 mice (heterozygotes) and 7.5% of the F_2 animals (heterozygotes and dominant homozygotes) had reticulum cell sarcomas at 52 weeks of age. By contrast, the incidence of reticular neoplasms was 23.8 %in homozygous recessive F_2 mice. These results seem best interpreted as indicative of a relationship between genotype and tumor incidence. On this basis, they are consistent with the hypothesis that susceptibility to development of reticulum cell sarcomas is influenced by the gene which controls the unique response of SJL/J mice to LDH virus infection.

While the primary defect is not known with certainty, it has been postulated that this gene has an effect on reticuloendothelial function[2]. For this reason, it seemed of interest to determine the effect of cyclophosphamide, a potent immunosuppressor with apparent abilities to impair the function of the reticuloendothelial system[8], on the development of reticulum cell sarcomas in F_2 mice.

The results obtained are presented in Table II. It can be seen that the tumor incidence was 15% in heterozygotes and dominant homozygotes and 45.5% in recessive homozygotes. Since these percentages are approximately twice those found in comparable groups of untreated F_2 animals (Table I), it would appear that treatment with cyclophosphamide resulted in a greater susceptibility to development of neoplasia. This observation is in agreement with the postulated mechanism of gene action as detailed above.

Zusammenfassung. Erste Hinweise, dass ein autosomales Gen, das die spezifische Reaktion von SJL/J-Mäusen auf Infektion durch das LDH-Virus kontrolliert, ebenfalls die Entwicklung von Endothelzellsarkomen beeinflussen könnte und zusätzlich eine Wirkung auf die retikuloendotheliale Funktion hat. Behandlung mit Zyklophosphamid hat eine Vermehrung von Tumoren in F_2-Mischlingen der Stämme SJL/J und BALB/c zur Folge.

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