New Aspects of Intestinal Carcinogenesis by 1,2-dimethylhydrazine dihydrochloride (DMH) and the Influence of Antilymphocyte Globuline (ALG) on its Progress*

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Summary. The induction of intestinal tumors by weekly subcutaneous administration of DMH and their metastatic spread, were studied in rats treated with or without ALG. Early morphologic changes of the intestinal mucosa were examined, as well as the influence of ALG on tumor induction, tumor growth, and mode of metastasis.

Whether treated with ALG or not, the intestinal mucosa showed
1. glandular hyperplasia with goblet cell depletion and elongation of the germinative zone (weeks 5—13),
2. glandular atrophy intermingled with hyperplasia (week 13),
3. induction of neoplastic micro-lesions (week 17),
4. production of macroscopic tumors (week 21).

Tumor induction was negative in 14% of rats (5/37) at the last autopsy after 33 weeks.

Particular attention was given to the morphologic changes of the mucosa over lymph follicles which was a frequent site of origin for neoplastic lesions.

In this study, ALG treatment failed to enhance tumor induction or tumor growth. It was ascertained, however, that ALG treatment significantly favors tumor spread by dissemination and conglomerate formation.

Key words: Intestinal Tumors — DMH — ALG — Metastases

It was often stated that impairment of the immune status may enhance the development of malignant neoplasms in the host. Supporting evidence was reported in humans (Allison, 1970; Penn, 1976) and in experimental animals (Miller et al., 1963; Johnson, 1968; Balner and Dersjant, 1969; Baroni et al., 1973), but negative results were also published (Wagner and Haughton, 1971). The divergence of findings may be attributed partly to the antigenicity of the tumors induced, and

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partly to the varying efficacy of the respective immunosuppressive agents, or of their administration schedule (Kroes et al., 1975).

We have to ask which process, during carcinogenesis, will be most liable to be affected by immunosuppression. The problem was discussed by several authors (Kroes et al., 1975; Balner and Dersjant 1969; Denengelse et al., 1976). The study of this subject is of relevance, too, in the search for an effective cancer treatment on the basis of immunological evidence, or to the understanding of the hazards of immunosuppressive therapy to man.

Our aim was 1. the morphological examination of the entire process of intestinal carcinogenesis induced by DMH in rats treated or untreated with ALG, and 2. a statistical analysis of the influence of ALG on each successive step of this carcinogenesis.

DMH was chosen as carcinogen because of its marked organotropy, safe carcinogenicity (Druckrey et al., 1967; Wiebecke et al., 1973) and, above all, its high frequency of metastases (Schauer et al., 1969; Wiebecke, 1975).

**Materials and Methods**

*Rats.* 120 ten-week-old male Wistar rats (colony-bred) were obtained from Zentralinstitut für Versuchstierforschung, Hannover (Germany). Their average initial body weight was 251 g. They were maintained on standard diet of Altromin in an air-conditioned room at 24°C. Water was given ad libitum. They were entered into the experiment after four days observation.

*Carcinogen.* 1% aq. solution of 1,2-dimethylhydrazine dichloride (DMH), purchased from Waldeck/Roxel, Germany, was administered once a week subcutaneously over 16 weeks in a weekly dosis of 20 mg/kg body weight. The total dosis for each rat was about 120 mg.

*ALG and NRG.* Rabbit anti-lymphocyte globulin (ALG) and normal rabbit globulin (NRG) were purchased from the Microbiological Association, Bethesda/Md, USA. 1 mg/0.5 ml aq. solution of ALG or NRG were given twice a week intraperitoneally over 13 weeks, starting one week prior to DMH administration. During the first week a double dosis was given. The total dosis was 28 mg per rat.

*Experimental Assay.* Table 1 demonstrates the experimental design. The rats were divided into four groups:

1. **Non-Serum Group.** 45 rats were given only DMH according to the above schedule. After the fifth week, no less than 2 rats were sacrificed every four weeks by random sampling, to examine the sequential changes of intestinal mucosa. 5, 10, and 15 rats were killed after 21, 29, and 33 weeks, respectively, for statistical comparison of the incidence and growth of malignant lesions, with those of the other groups.

2. **ALG Group.** 45 rats were given both DMH and ALG. They were partly sacrificed for testing according to the schedule described for the non-serum group.

3. **NRG Group.** 15 rats were given both DMH and NRG, of which 10 were sacrificed after the 33rd week.

4. **Control group.** 15 rats were maintained as untreated controls, of which 10 were sacrificed after the 33rd week.

Clinical symptoms of each rat were checked every other day. Body weight was recorded every four weeks. Rats which died spontaneously during experiment, were autopsied and added to the analysis for carcinogenesis. Peripheral lymphocyte counts were monitored at days 14, 15, 16, and 17 of the experiment during ALG treatment to examine its effect.