Studies on Eosinophil Granulocytes. II. The Effect of Cytostatics on the Uterine Response to Oestrogen

The growth of the uterus of the spayed mouse upon oestrogen stimulation is accompanied by an intense eosinophilia, which accounts for the 'uterine peroxidase'[1]. This occurs also in the rat. The eosinophilia is largely, but the growth only slightly, depressed by the simultaneous administration of progesterone or cortisone[2]. In the rabbit uterus no peroxidase activity is evoked by oestrogens under any condition[1]. There are thus examples that oestrogens can induce growth of a uterus without a concomitant eosinophilia or with only a slight eosinophilia in the organ.

The intention of the present investigation was to study the weight response of the uterus of the spayed mouse to oestrogen in mice with a low number of available eosinophils.

Methods and materials. A depression of the eosinophils could be obtained only as part of a general depression of granulocytopoiesis. This was brought about by treating the animals before and during the action of the oestrogen with drugs which are potential depressors of hematopoiesis. Since such a treatment would interfere with a variety of other functions, several substances were employed, most of which are well-known cytostatics[3]. Aminotriazole reduces the amount of catalase in the liver within a few hours[4] and inhibits thyroid ^{131}I uptake and the organic binding of iodine without affecting the iodide trap[5].

Very heavy dosages were used to ensure an effect on hematopoiesis, and some animals died. It seemed unfeasible to attain a stronger average effect on a group, and regular leucocyte counts were not performed. The drugs were dissolved or suspended in saline and injected i.p. between the hours 0900 and 1000. 17β-oestradiol *p*-propoxyphenyl propionate in peanut oil was used as a long-acting oestrogen and given s.c. in a single injection.

To supply sufficient quantities of a homogeneous material for all analyses, the uteri were weighed individually, pooled, and homogenized with 11 parts (v/w) of 20 m*M* sodium phosphate, pH 7.0. Dry weights, corrected for ash, were determined in duplicate (error in single determination $\pm$ 1.6%), peroxidase activities in triplicate (15, 30 and 45 μl, mean variance of residue 0.008 $\pm$ 0.005), and DNA-P and RNA-P in duplicate (errors in single determinations $\pm$ 1.7 and $\pm$ 2.6%). For the assay of catalase, liver pieces of approximately equal size from all animals in a group were pooled, weighed, and homogenized with water, 100 mg/2 ml. The activites were determined[6] in duplicate (error in single determination $\pm$ 17%) and recalculated to give the velocity constant/g of wet weight/sec. Other methods and materials were described in the previous communication[1]. 8 animals were started in every group.

The duration of the joint exposure to a drug and the oestrogen was chosen so as to exceed the regeneration time of the eosinophils. The life span of the eosinophil in normal rats has been estimated as 4 days in the bone marrow, a few hours or less in the blood stream, and 2–7 days in the tissue[7]. The weight response of the uterus to oestrogen under the present conditions follows a logarithmic course, and the weight reaches its full value in 4 days[1].

Results and comments. The general condition of the mice deteriorated in most groups during the 15 days of drug administration, markedly so in the mercaptopurine, fluorouracil, and metothrexate groups. The lower gain in weight of the uterus in comparison to that in the saline group may to some extent be a consequence of the general deterioration.

In all groups the uterus responded to the oestrogen with growth and with eosinophilia, in spite of the very heavy dosages and the long duration of the experiment. Some deviations from the saline group values occurred (Table). 3 drugs significantly depressed the peroxidase activity in the uterus, and 4 drugs hampered the gain in

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The effect of some drugs on uterine weight and peroxidase activity and on liver catalase. The mice were spayed on day – 11, received the drug in daily injections from day – 4 on, the long-term oestrogen in a single injection on day 0, and were sacrificed on day 11. All groups except 'Nil' were given 3 μg long-term oestrogen in addition to the listed drug. *Italics denote more than 95% probability for a difference from the saline group*

Drug mg/day/mouse		Uterus Wet weight mg	Homogenate mg dry weight/ml	Peroxidase activity ΔA_{470}/min/mg dry weight	Liver catalase k/g wet weight/sec
Nil		9.2 $\pm$ 1.6	17.0	0	
Saline		95.9 $\pm$ 28.2	13.3	0.51 $\pm$ 0.07	1.7
Desoxypyridoxine	1.5	*64.8 $\pm$ 15.9*	14.3	0.44 $\pm$ 0.07	1.3
Aminotriazole	0.5	*74.5 $\pm$ 23.9*	12.9	0.57 $\pm$ 0.10	1.3
Streptomycin	1.0	87.5 $\pm$ 29.6	12.0	*0.29 $\pm$ 0.01*	1.6
6-Mercaptopurine	0.5[a]	*68.7 $\pm$ 31.5*	13.4	*0.25 $\pm$ 0.02*	*0.6*
5-Fluorouracil	0.2[a]	*62.7 $\pm$ 7.3*	13.6	0.50 $\pm$ 0.01	*0.7*
Metothrexate	0.03[b]	83.7 $\pm$ 25.2	12.6	*0.71 $\pm$ 0.08*	1.2
Chloramphenicol	2.8	94.6 $\pm$ 13.3	11.5	*0.38 $\pm$ 0.01*	1.0

[a] 1 animal died; 4 out of 8 animals died with twice the dosage. [b] 2 animals died.

weight. There is, however, no obvious correlation between the 2 effects, and presence or availability of the eosinophils seem to be no prerequisite for the response in total weight of the mouse uterus to the long-acting oestrogen. The slightly elevated peroxidase activity in the metothrexate group was unexpected and is difficult to explain unless it is a random effect. Oestradiol is known to protect hematopoiesis against metothrexate[8].

The liver catalase was chosen as representing an organ with a marked ability for regeneration, being a hydroperoxidase itself. The catalase activities were reduced in the mercaptopurine and fluorouracil groups, but with no obvious correlation to the other 2 parameters. The normal value with aminotriazol may be accounted for by the duration of the experiment.

DNA-P and RNA-P for the saline group were found as 2.3 and 3.4 μg/mg dry weight. No group showed significant deviations, the ranges being 2.0–2.7 (means 2.4 ± 0.2) and 3.0–3.7 (means 3.3 ± 0.2) respectively[9].

Zusammenfassung. Bei kastrierten, weiblichen Mäusen, die verschiedene Cytostatica zusammen mit einem langwirkenden Oestrogen erhielten, wurde Gewichtszunahme und Eosinophilie des Uterus nach 11 Tagen gemessen. Zwischen den beiden Wirkungen bestanden offensichtlich keine Beziehungen. Das DNA/RNA Verhältnis blieb unverändert.

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Die Induktion lysogener Bakterien durch Nitrosamide

Im Rahmen von Untersuchungen über die Beziehungen zwischen Cancerogenese und Lysogenie hatten wir früher die Wirkung der sehr aktiven Cancerogene Dimethylnitrosamin und Diäthylnitrosamin geprüft[1]. Beide Substanzen vermochten *Escherichia coli* K12 (λ)-Zellen nicht zu induzieren und erwiesen sich bei *Serratia marcescens* – wie auch bei *Neurospora crassa*[2] – als nicht mutagen. Wir führten dies darauf zurück, dass in Mikroorganismen Diazoalkan bzw. Carbonium-Ionen als die eigentliche alkylierende «Wirkform» der Nitrosamine, die für die toxische und mutagene Wirkung dieser Substanzen beispielsweise bei *Drosophila melanogaster*[3] verantwortlich sind, mangels entsprechender Enzyme nicht gebildet wird.

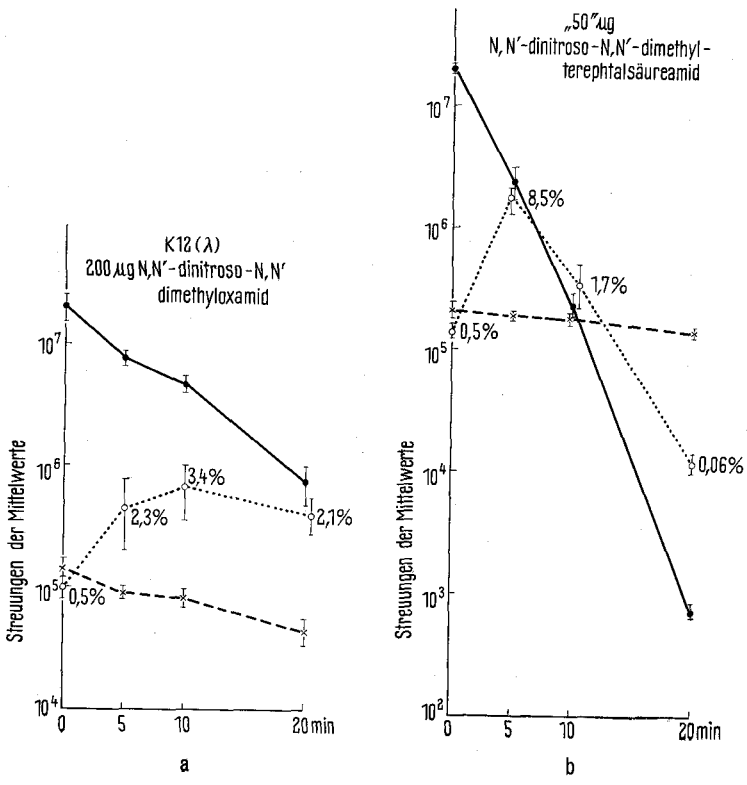

Fig. (a) und (b). Die Wirkung von N,N'-dinitroso-N,N'-dimethyl-oxamid (DDO) (a) und N,N'-dinitroso-N,N'-dimethylterephthalsäureamid (NMT) (b) auf *Escherichia coli* K12 (λ)-Populationen in semisynthetischem (SS-) Medium. Von NMT waren 50 μg/ml eingewogen worden, wurden aber nicht vollständig gelöst. Mittelwerte aus 6 (a) bzw. 4 (b) Versuchen. ●—● = lysogene Bakterien (Koloniebildner), x-----x = freie λ-Phagen, o·····o = induzierte Bakterien (Plaque-Bildner).