Virchows Arch. A Path. Anat. and Histol. 376, 117–132 (1977)

Virchows Archiv A

Diaplacental Carcinogenesis: Tumor Localization and Tumor Incidence in NMRI Mice after Diaplacental Initiation with DMBA and Urethane and Postnatal Promotion with the Phorbol Ester TPA in a Modified 2-Stage Berenblum/Mottram Experiment

Klaus Goerttler and Heinz Löhrke

Institute of Experimental Pathology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, Federal Republic of Germany

Summary. Diaplacental initiation with the carcinogens DMBA or urethane followed by repeated topical treatment of mice of the F_1 generation with the tumor promoter TPA leads to the formation of benign and malignant tumors on the skin of the back as well as in other tissues and organs. The tumor yield in this modified 2-stage Berenblum/Mottram experiment considerably exceeds the number of spontaneously formed tumors and of tumors produced by initiation alone. Further differences can be demonstrated in the malignancy rate, the formation of multiple tumors in various organs, additional non-neoplastic alterations and in a reduction of the lifetime of the animals. The effect of the tumor promoter TPA is not restricted to carcinogenesis in the back skin. Obviously, TPA is able to activate inititated tumor cells in internal organs to form tumors. This, in turn, implies the absorption of the substance via the blood vessels and its distribution throughout the body. The preferential occurrence of tumors in the genital tract of female mice (carcinomas and sarcomas of the vaginal wall, granulosa cell tumors of the ovaries) points to a possible hormonal involvement; in this context, relevance to prenatally induced tumors in human pathology is discussed. The results emphasize the important role of prenatal carcinogenesis and indicate the increased risk to man by either prenatal initiation or postnatal promotion.

Key words: Carcinogenesis – Cocarcinogenesis – Diaplacental – Organs involved – Multiple tumors – DMBA initiation – Urethane initiation – Phorbol ester promotion – Autopsy data – Survival rates.

Introduction

In 1971, Herbst et al. reported the occurrence of vaginal adenocarcinomas in daughters of women who had received synthetic oestrogens (diethylstilboestrol)

For offprints contact: Prof. Dr. K. Goerttler

during pregnancy in order to prevent spontaneous abortion. Subsequently, these observations have been repeatedly confirmed (Herbst et al., 1975) and interest in and relevance of earlier experiments on diplacental carcinogenesis have considerably increased (Mohr and Tomatis, 1973; Ivankovic, 1975). Experiments from our laboratory showed that the 2-stage Berenblum/Mottram experiment (initiation-promotion) was fully effective by either transmaternal or trans(dia)placental route: Initiation (step I) could be induced via mothers milk or by direct transfer through the mother animal (Goerttler and Löhrke, 1976a, 1976b). Subsequent postnatal promotion (step II) with a cocarcinogen (i.e. topically applied TPA) led to a high tumor yield and the early expression of tumors in the epidermis. Application of only one of the substances at the same dose level did not lead to the formation of tumors within an investigation period of 52 weeks. The highest skin papilloma yield along with additional transformation into squamous cell carcinomas was obtained after DMBA-initiation from prenatal days 16 to 19. The highest total tumor yield was obtained with mice initiated with DMBA from the 18 to 20 day of fetal life.

Unexpectedly, and beyond the scope of the experiment, we also observed a high incidence of tumors of various internal organs (Goerttler and Löhrke, 1976b). Therefore, beside the purely local effect of TPA a more general promoting property of this substance must be taken into consideration. The original experiment, limited to an investigation period of 52 weeks, but resolution of new problems evident in the course of the experiment was not possible. We therefore decided to keep the animals alive without further treatment and to summarize the results of the post-mortem examinations in the present paper. The new findings give further insight into diaplacental carcinogenesis.

Material and Methods

The original experimental set-up has previously been described in detail (Goerttler and Löhrke, 1976b). Female mice were vaccinated against ectromelia. Animals were paired at 12 weeks; females with positive vaginal smears (spermatozoa) were randomly divided into groups and treated as indicated in Table 1. Table 1 also shows the number of abortions per group of mother animals and the number of mice of the F_1 generation. At the end of the sucking period the young animals were separated from their mothers and assigned to subgroups a and b. Between the ages of 12 and 36 weeks the animals of subgroups 1a–8a were treated twice weekly with an acetone solution of the tumor promoter TPA[1] (12-0-tetradecanoyl-phorbol-13-acetate). This represents a total of 48 applications to the back skin of the mice of 0.01 µm mole TPA = 0.00615 mg TPA in 0.1 ml acetone. Animals of subgroups 1b–8b received only acetone. Statistical significance of the reported values was calculated according to Gabriel (1966). With regard to the survival times in the individual groups, the medians with the corresponding 5% confidence intervals were determined. Furthermore the groups were checked for global median differences by means of the Kruskal-Wallis Test (Kruskal, Wallis, 1952). Finally, all pairs of groups were compared by multiple comparisons according to Dunn (1964) using a significance level of $\alpha = 0.05$[2].

[1] The substance was kindly provided by Prof. E. Hecker, Institute of Biochemistry, German Cancer Research Center

[2] We are grateful to Dr. Zentgraf, Department of Experimental Design, Institute of Documentation, Information and Statistics, German Cancer Research Center

Table 1. Experimental set-up. Time of application of the carcinogens DMBA and urethane, respectively. Number of pregnant mother animals, number of abortions and of animals of the F1 generation. Assignement of the F1 animals to subgroups a or b, indicating treatment with the phorbol ester TPA or ommission of this treatment

Group	Substance and kind of application	Dosage (kg body-weigth)	Gestation days	Number of pregnant mother animals[a]	Abortions	Number of animals of the F1 generation	Number of F1 animals assigned to subgroup a or b (with or without TPA treatment	
							a	b
1	Control; livio-oil® oral	1 ml	14–21	6	0	56	40	16
2	DMBA-oral	5 × 15 mg = 75 mg	14–18	9	3	50	40	10
3	DMBA-oral	5 × 30 mg = 150 mg	14–18	19	14	48	38	10
4	DMBA-oral	4 × 15 mg = 60 mg	16–19	5	0	45	35	10
5	DMBA-oral	4 × 15 mg = 60 mg	17–20	5	0	44	34	10
6	DMBA-oral	4 × 15 mg = 60 mg	18–21	5	0	50	40	10
7	Urethane i.p.	3 × 60 mg = 180 mg	17–19	5	1	32	22	10
8	Urethane i.p.	3 × 60 mg = 180 mg	18–20	5	1	30	20	10

[a] The fate of the mother animals is not reported in this paper. All data, including histological findings of post-mortem examination of the animals are available. In comparison with the hitherto known alterations, they don't provide any further information. However, the observed survival times are essentially higher than those of the animals of the F1 generation

Results

1. Tumor Incidence, Localization, Benign and Malignant Tumors

Table 2 shows the number of tumor-bearing animals in each group and also gives the number of malignant tumors of different body sites. Whereas 233 of 237 animals of groups 2–6 were tumor-bearers, only 16 of 40 animals of the non-initiated, TPA-treated subgroup 1a had developed tumors. Among 16 mice of the untreated control group 1b 6 had tumors. Initiation with

Table 2. Diaplacental initiation and postnatal promotion. Number of benign and malignant tumors; localisation of malignant tumors

Subgroup	Number of animals	Number of tumor-bearing animals	Localisation of malignant tumors								
			Back skin	Lung	Liver	Ovary	Neurogenic tumors	"Other organs"[a]	Leukemias	Total (without leukemias)	Total (with leukemias)
1a	40	16	–	–	–	–	–	–	5	–	5
1b	16	6	–	–	–	–	–	–	5	–	5
2a	40	40	7	12	2	6	2	2	9	31	40
2b	10	9	–	5	–	1	–	–	1	6	7
3a	38	38	13	2	7	5	1	9	10	38	48
3b	10	10	–	4	1	4	–	–	1	9	10
4a	35	35	10	8	4	5	–	7	9	34	43
4b	10	9	–	3	1	–	–	1	1	5	6
5a	34	34	8	8	3	3	–	2	5	24	29
5b	10	10	–	–	4	2	–	2	–	8	8
6a	40	38	7	12	5	1	–	1	7	26	33
6b	10	9	–	3	–	–	–	–	2	3	5
7a	21	17	4	2	7	–	–	1	2	14	16
7b	10	7	–	4	1	–	–	–	1	5	6
8a	20	18	2	3	3	–	–	1	1	9	10
8b	10	6	–	1	1	1	–	–	–	3	3

[a] The malignant tumors in "other organs" refer to:
Subgroup 2a: vagina (1×), cranium (1×)
Subgroup 3a: vagina (2×), endometrium (1×), Harderian gland (1×), seminal vesicle (1×), paranasal cavity (1×), foreleg (1×), anal region (1×), adrenal medulla (1×)
Subgroup 4a: vagina (1×), endometrium (1×), testis (1×), small intestine (1×), kidney (1×) adrenal medulla (1×)
Subgroup 4b: forestomach (1×); subgroup 5a: colon (1×), rectum (1×); subgroup 5b: paranasal cavity (1×), lymph nodes (1×)
Subgroup 6a: mammary gland (1×); subgroup 7a: mammary gland (1×); subgroup 8a: endometrium (1×)

urethane instead of DMBA turned out to be less effective with respect to tumor formation (48 of 61 animals of groups 7 and 8). Whereas 185 of 187 of initiated and TPA-treated animals of subgroups 2a–6a showed tumors, only 35 of 41 of groups 7 and 8 were tumor-bearers. Initiation with DMBA alone proved to be almost equally active. Forty-seven of 50 animals of sub-groups 2b–6b developed tumors; in contrast, urethane initiation without subsequent promotion caused formation of tumors in only 13 of 20 animals of subgroups 7b and 8b. With regard to the total number of animals with benign and malignant tumors there seem to be no difference between animals treated according to the 2-stage application scheme and animals which were only initiated with DMBA. However, the apparently high conformity of the results in the a- and b-groups in our experiment is misleading. If malignant tumors only are grouped together it becomes evident that skin carcinomas developed exclusively after the combined treatment schedule (either DMBA/TPA or urethane/TPA; sub-

groups 2a to 8a). In the two control groups 1a and 1b only leukemias were observed. Regarding the number of malignant tumors, all a-groups show significant differences (significance level = 0.005) when compared with the control groups 1a and 1b. Table 3 summarizes the incidence and localization of benign tumors up to the death of the animals. As expected, tumors of this type could be demonstrated on the back skin only after initiation and promotion. A tendency to develop spontaneous lung adenomas has already been observed in the NMRI strain used in the experiment. However, the incidence of these tumors in subgroups 2a–8a far exceeded the values reached in subgroups 1a and 1b. No difference could be seen between the a- and b-groups. On the other hand, 36 of 187 animals of subgroups 2a–6a showed liver tumors whereas this kind of tumor was seen in only 6 of 50 animals of subgroups 2b–6b. The corresponding data for all animals of groups 2a–8a are 229/49 and 70/8 for subgroups 2b–8b, respectively, which means that the proportional number of animals with benign liver tumors has doubled. Potentially precancerous conditions could be demonstrated in the back skin (hyperplasia), the liver (cholangiosis), the

Table 3. Diaplacental initiation and postnatal promotion. Number and localisation of benign tumors

Subgroup	Number of animals	Back skin	Lung	Liver	Ovary	Harderian gland	"Other organs"[a]
1a	40	–	6	–	1	1	3
1b	16	–	2	–	–	–	–
2a	40	14	28	3	–	–	3
2b	10	–	7	–	–	1	–
3a	38	14	24	12	2	1	2
3b	10	–	3	2	–	–	1
4a	35	15	20	10	–	3	7
4b	10	–	5	1	1	1	–
5a	34	8	20	5	1	8	3
5b	10	–	5	1	–	1	–
6a	40	18	4	6	–	2	2
6b	10	–	7	2	–	1	1
7a	22	4	10	8	–	–	–
7b	10	–	4	–	2	–	1
8a	20	6	12	5	–	–	1
8b	10	–	5	2	–	–	1

[a] The benign tumors in "other organs" refer to:
Subgroup 1a: uterus (1×), anal gland (1×)
Subgroup 2a: anal gland (1×), bronchus (1×), rectum (1×), uterus (1×)
Subgroup 3a: uterus (1×)
Subgroup 4a: epididymis (1×), upper lip (1×), seminal vesicle (1×), spleen (1×), forestomach (1×), uterus (1×), endometrium (1×)
Subgroup 5a: spleen (1×), anal gland (1×), adrenal medulla (1×)
Subgroup 6a: thyroid gland (1×), forestomach (1×)
Subgroup 6b: vagina (1×)
Subgroup 7b: oesophagus (1×)
Subgroup 8a: anal gland (1×)
Subgroup 8b: spleen (1×)

ovaries (cysts), the vagina (fibrosis of the wall), the endometrium (glandular and cystic hyperplasia), the Harderian gland (hyperplasia), and the anal gland (hyperplasia, cystic transformation). Hyperplasia was a typical finding in TPA-treated animals of the a-groups and was virtually absent in the b-groups. This part of the investigation may be summarized as follows:

(a) The number of tumor-bearing animals is significantly higher after initiation and promotion when compared with the untreated controls as well as with TPA-treated controls. No statistically significant differences can be shown between the a- and b-groups (initiation/promotion versus promotion).

(b) The number of animals with benign lung tumors is higher in animals which had been either initiated or initiated and promoted than in the control groups 1a and 1b, although these animals too showed small numbers of spontaneous lung tumors. There are no differences between the initiated and the initiated and promoted animals.

(c) Animals with benign liver tumors were only observed in subgroups 2a–8a and 2b–8b. Initiation plus promotion resulted in a doubling of the percentage of liver tumors when compared with initiation alone.

2. Incidence of Single and Multiple Tumors, Organs Involved, Exceptional Cases

In Table 4 data are presented on the number of animals with benign and malignant tumors. It should be emphasized that the data only refer to the numerical participation of different organs or tissues per experimental group rather than to the number of tumors in the organs under consideration. Whereas 144 of a total of 269 animals of all a-groups (including 40 animals of group 1a) showed multiple tumors (ratio 1:0.54) only 19 of 86 animals of the b-groups had developed tumors in several organs (ratio 1:0.22). In neither instance did multiple tumors occur in the two control groups 1a and 2a (TPA treatment alone). (A thymus lymphoma combined with lympho-sarcomatosis of several lymph-nodes in group 2a was not considered to be a multiple tumor). The overall incidence of malignant and also of benign multiple tumors in the initiation/pro-

Table 4. Number of animals with benign and malignant multiple tumors in subgroups a and b (initiation/promotion; initiation alone)

Subgroup a	Number of animals	Malignant tumors	Benign tumors	Subgroup b	Number of animals	Malignant tumors	Benign tumors
1a	40	–	–	1b	16	–	–
2a	40	7	13	2b	10	2	1
3a	38	12	16	3b	10	2	1
4a	35	15	18	4b	10	2	–
5a	34	8	11	5b	10	3	2
6a	40	5	18	6b	10	–	2
7a	22	6	6	7b	10	1	2
8a	20	2	7	8b	10	1	–

motion groups may be expressed by the ratio 1:0.24:0.55, that is total number of animals:number of malignant:number of benign primaries. In contrast, the initiation groups showed a corresponding ratio of 1:0.16:0.11, thus clearly indicating a preponderance of the tumor yield in our modified 2-stage Berenblum/Mottram experiment.

Two further points were of interest. First, are there variations in the number of animals without any tumors or potentially precancerous conditions in the individual groups, and second, are there differences with regard to the number of organs with primary tumors (not metastases) within the groups? Table 5 clearly shows that there are significant differences between control groups 1a/b and subgroups 2a–8a and 2b–8b, respectively. Within the experimental groups the combination DMBA/TPA was constantly more effective than the combination urethane/TPA. Furthermore, there are differences between the subgroups a and b with respect to the spectrum of neoplastically transformed organs: whereas the combination DMBA/TPA led, on an average, to tumor formation in 13 different organs, DMBA initiation alone influenced only 5 organs. The combination urethane/TPA was effective in approximately 5 organs; urethane initiation alone gave rise to tumor formation in 2–3 organs in the corresponding subgroups. TPA treatment alone produced tumors in 6 organs whereas only 3 organs were involved in the untreated controls. In subgroup 1a, 5 leukemias, 6 lung adenomas, 1 hemangioendothelioma of one uterine horn, 1 adenoma of the ovary, 1 adenoma of the Harderian gland, and 2 adenomas of the anal gland could be demonstrated. Analysis of subgroup 1b revealed 2 leukemias, 1 thymic lymphoma combined with lymphosarcoma of the lymphnodes, 2 lung adenomas, and 1 hemangioma of one ovary. These findings, however, must be seen in relation to the longer survival time of the animals in both groups (see also Table 6).

There was not only a broader variety of different tumor-bearing organs in the a-subgroups but also different neoplasias in the involved organs them-

Table 5. Number of animals without hyperplasiogenic, facultative preneoplasias; number of tumor-bearing organs in the individual groups

Subgroup a	Number of animals	Number of animals without hyperplasias and tumors	Number of organs with primary tumors	Subgroup b	Number of animals	Number of animals without hyperplasias and tumors	Number of organs with primary tumors
1	40	13	6	1	16	7	3
2	40	1	13	2	10	1	4
3	38	–	17	3	10	–	5
4	35	–	16	4	10	–	6
5	34	–	12	5	10	–	6
6	40	–	9	6	10	–	4
7	22	–	5	7	10	2	5
8	20	2	6	8	10	3	4

selves. In the liver for instance, beside hepatocellular carcinomas, adenomas and hyperplastic lymph nodes, there were also cholangiomas, cysts, and hemangioendotheliomas. In the back skin in addition to carcinomas follicular as well as anaplastic squamous cell carcinomas and papillomas, fibroepitheliomas, acanthopapillomas, and rarely, adenomas of the sebaceous glands, also cysts, hyperplasias and subcutaneous sarcomas could be demonstrated. Pathological alteration in the vagina included not only simple fibrosis of the vaginal wall together with replacement of the muscular layers by connective tissue (fibrous tissue or poor) but also leiomyosarcoma and undifferent-celled sarcoma. In one case we found a mixed tumor consisting of sarcomatous and adenomatous parts (Fig. 1).

Among the organs in which tumors could be demonstrated, the back skin was regularly involved in the 2-stage experiment. Frequently, the lungs and the blood-forming tissues participated in tumor formation in both the controls and treated animals. In the 2-stage experiment, the liver and the ovaries repeatedly showed tumors whereas initiation alone was less effective in these organs. The alterations in the endometrium were predominantly glandular and cystic hyperplasias together with ovarian tumors. Some exceptional cases are described below. Occasionally, malignant and some benign neoplasms occurred in the nervous system, meninges, subcutaneous tissue, skeleton, and the vaginal wall. We observed neurinomas, malignant neurilemmomas and a meningial sarcoma. Tumors of this type are rarely encountered in mice after diaplacental initiation; in contrast, they are frequently, if not regularly, produced in rats. Sarcomas were seen in the subcutaneous adipose tissue and developed from periosteal origins in the bones of the extremities, of the thorax and the cranium. A detailed description of the various combinations would not be within the bounds of this discussion. However, some cases of particular interest will be mentioned briefly. In subgroup 2a, one female which died at the age of 10 months showed the combination of the following different tumors: papillary mammary gland carcinoma, liver hemangioendothelioma, multiple adenocarcinomas of the lung with histologically detectable transition to an adenocancroid, a highly anaplastic granulosa cell tumor of one ovary and also an adenosarcoma of the vaginal wall (Fig. 1). In subgroup 4a, one instance of a leiomyosarcoma originating from the vaginal wall and one case of a sarcoma of stromal origin of the endometrium, a granulosa cell tumor of the ovary, a lung carcinoma, and a papilloacanthoma of the back skin were demonstrated. One female showed the combination of a granulosa cell tumor of the ovary metastasizing to liver and kidneys, with a sarcoma of stromal origin of the endometrium (Fig. 2). A female of subgroup 2a, which died at the age of 16 months, showed a macroscopically detectable deformed cranium together with the following histological alterations: one small papillary fibroepithelioma of the back skin, multiple trabecular lung adenomas with transition into follicular and trabecular adenocarcinomas, glandular and cystic hyperplasia of the endometrium, fibrosis of the vaginal wall, and leiomyomatosis of the adjacent uterine horn, one dysontogenetic tumor and a cyst of one ovary, a big, malignant granulosa cell tumor of the other ovary, fibrous dysplasia of the cranium with transition into a sarcoma of periosteal origin, which infiltrated the orbital wall and the periorbital

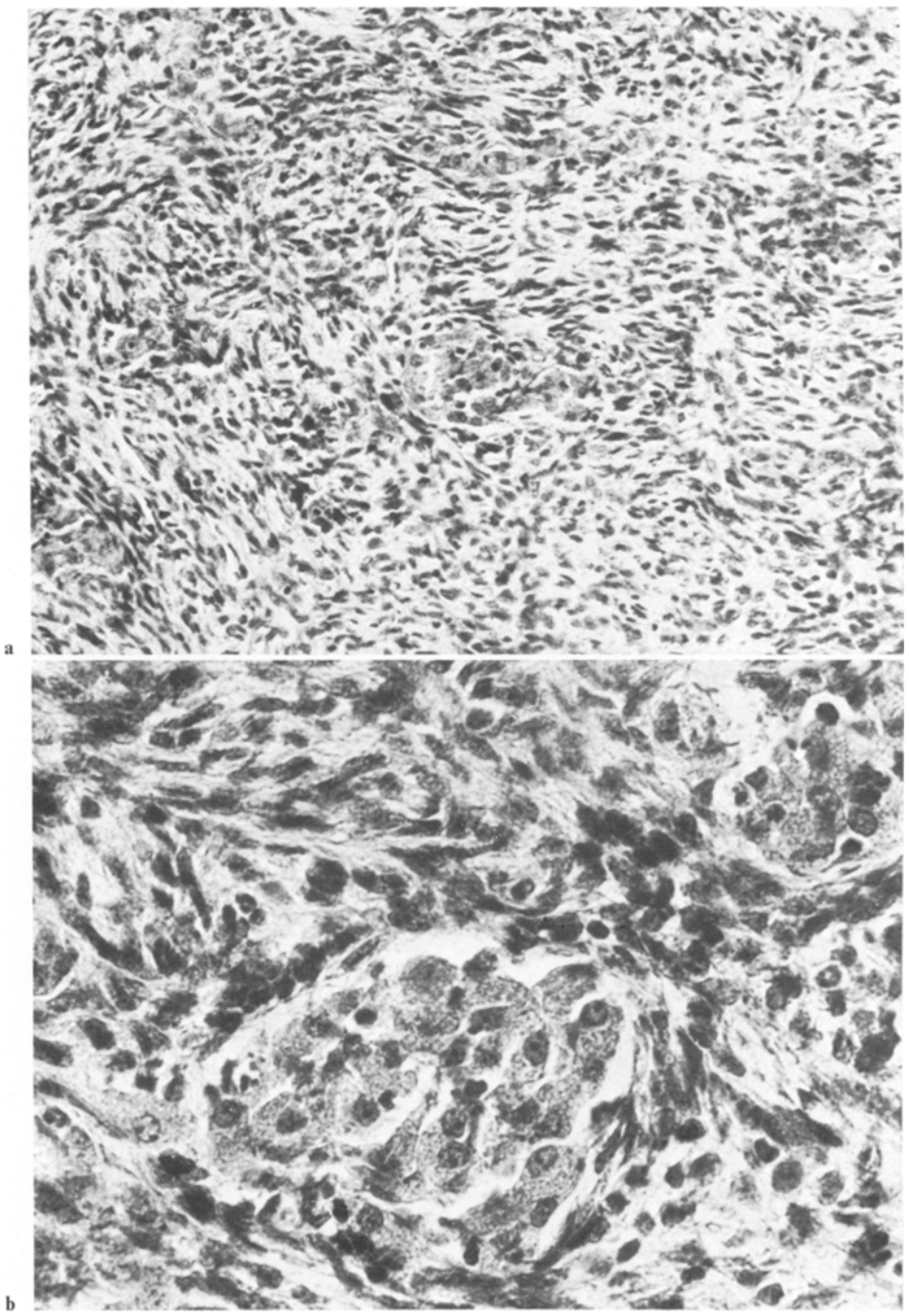

Fig. 1a and b. Sarcoma of the vaginal wall. In **a** (magn. 40×) the preponderance of the sarcoma is visible. In **b** (magn. 100×) a single adenomatous part is shown. Subgroup 2a, specimens no. 1093/76

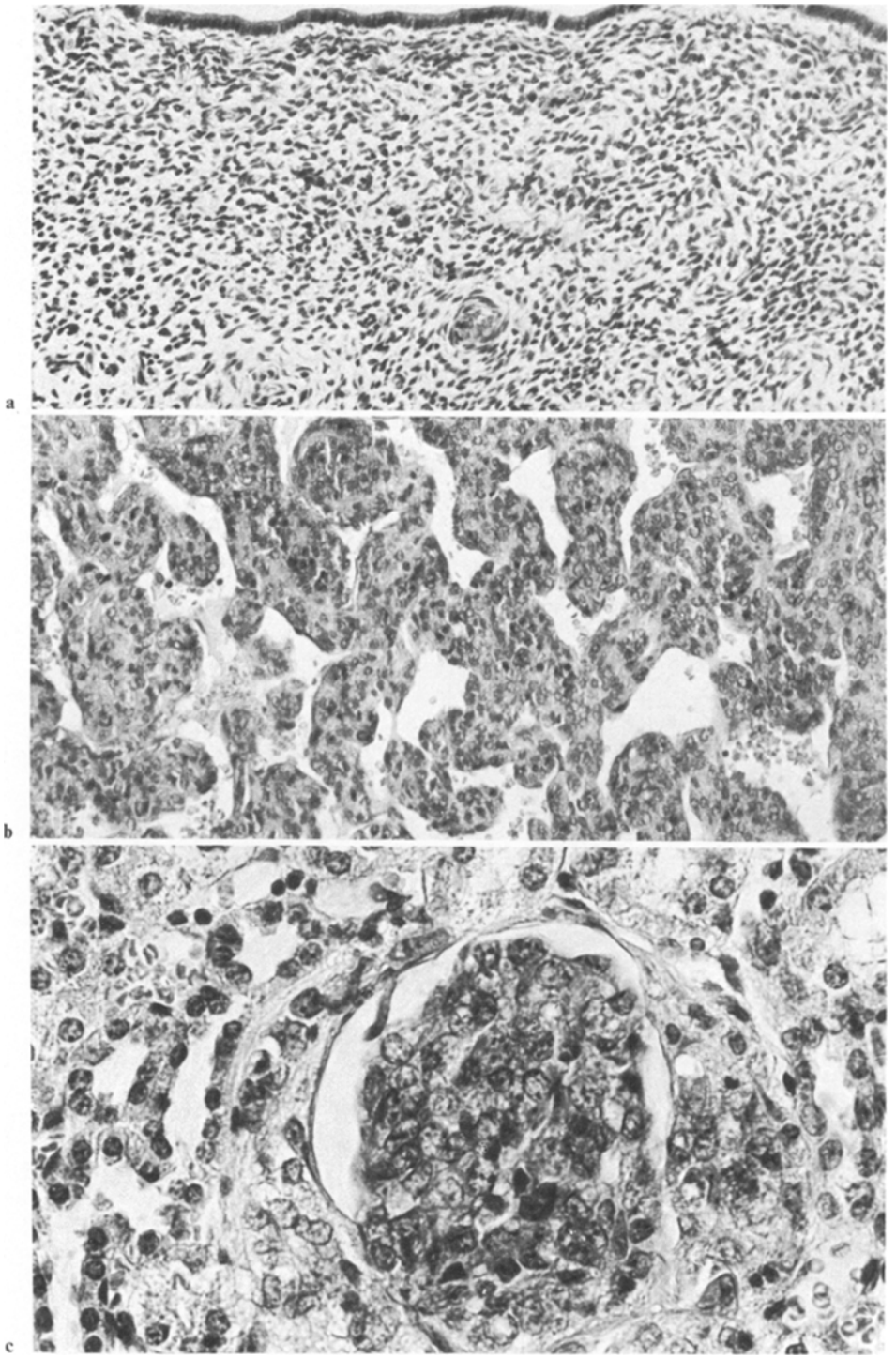

Fig. 2a–c. Sarcoma of endometrial origin in an uterine horn (**a**), (magn. 40×), combined w metastasizing granulosa cell tumor of the ovary (**b**), (magn. 40×). A micrometastasis in a glomerul is shown in **c**) (magn. 100×). Subgroup 4a, specimens no. 265/76

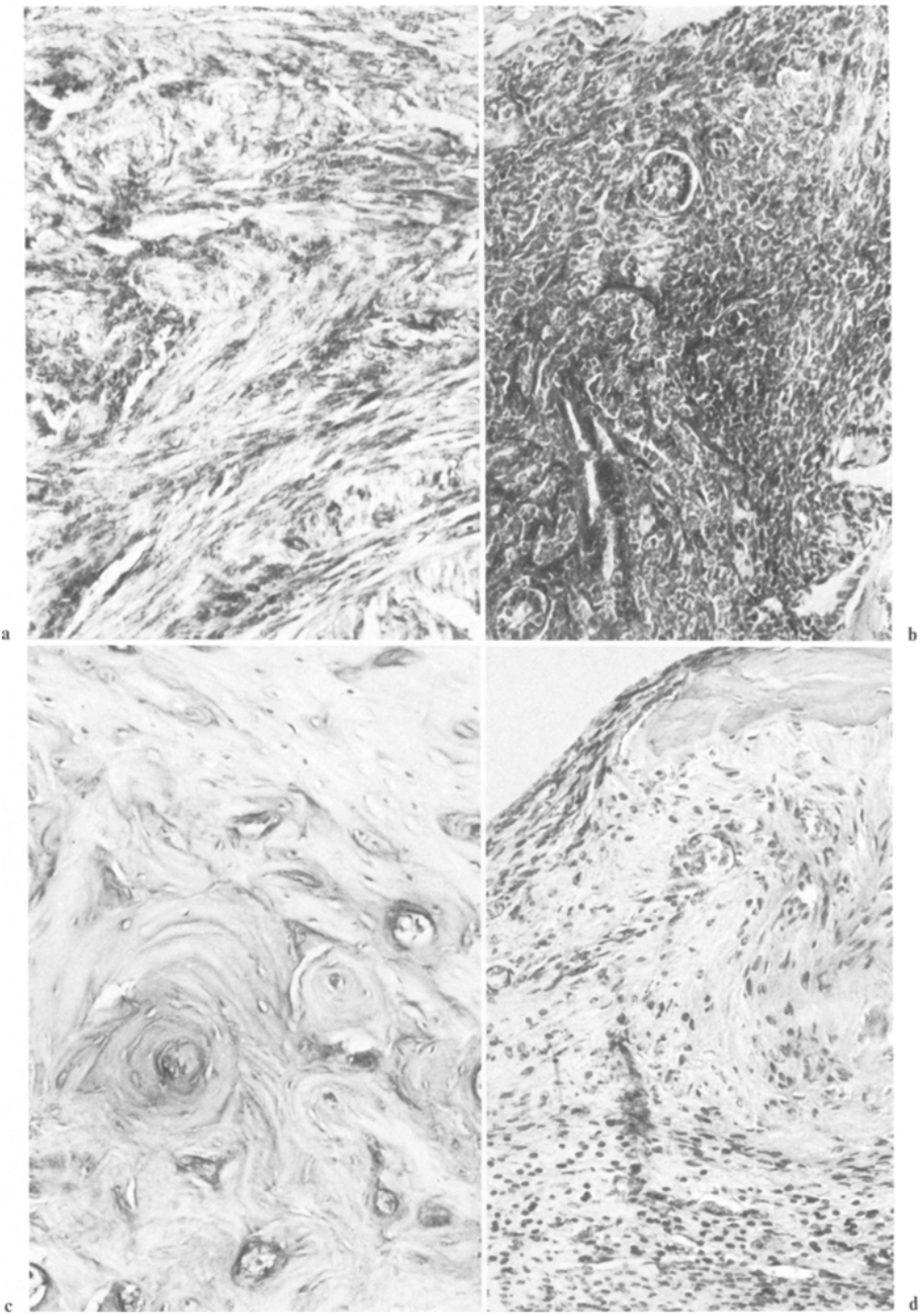

Fig. 3a–d. Borderline case between oncology and tetralogy: Leiomyoma uteri (**a**), combined with a dysgenetic tumor of the rete ovarii (**b**), fibrous dysplasia of the cranial vault (**c**), and periosteogenic sarcoma of the cranium (**d**). All magnifications 40×. For other tumors of this case see text. Subgroup 2a, specimens no. 2204/76

Table 6. Median values and confidence intervals of the survival times in the individual groups[a]

Subgroup a	Medians	Confidence intervals	Subgroup b	Medians	Confidence intervals
1	654	603–689	1	643	595–752
2	354	308–392	2	302	248–364
3	306	245–364	3	420	168–490
4	392	357–420	4	402	231–516
5	369	327–406	5	448	315–511
6	424	336–448	6	459	301–525
7	494	385–623	7	525	280–658
8	459	357–560	8	516	369–593

[a] With respect to the survival times of the mother animals, see also Table 1.

connective tissue and hyperplasia of the Harderian gland (Fig. 3). This case may be thought to represent a boundary line case between oncology and teratology.

3. Survival Rates

In Table 6 the median values of the survival times of the animals in the individual groups are summarized. As expected, animals in control groups 1a and 1b showed the longest survival times of 654 and 643 days, respectively. The survival time of the DMBA/TPA-treated animals of subgroups 2a–6a was significantly shortened when compared with control group 1a. Similarly, the urethane/TPA subgroup 8a differed from subgroup 1a, whereas subgroup 7a did not. In the carcinogen-treated b-groups significant differences in the survival times were observed between subgroup 1b and subgroups 2b–6b. Animals treated only with urethane (subgroups 7b and 8b) showed no differences. TPA treatment alone did not lead to a reduction in the survival time. In comparison with the DMBA/TPA- and DMBA-groups, survival time was higher in the urethane/TPA- and urethane-groups. The shortest survival time was seen in groups 2 and 3 in which the mother animals had received 75 and 150 mg DMBA, respectively, between days 14 and 18 of pregnancy. However, it should be remembered that group 3 showed by far the highest abortion rate and thus a considerable reduction in the number of offspring. Only minor differences could be seen between the DMBA/TPA- and the TPA-groups. With the exception of group 2b the DMBA-initiated animals showed a slightly longer survival time.

Discussion

The modification of the classical 2-stage Berenblum/Mottram experiment described in this paper (prenatal initiation, postnatal promotion) led to results

which are important in several aspects. The anticipated formation of skin tumors was observed in the initiated and subsequently promoted animals within an investigation period of 52 weeks, a fact which demonstrates the increased effectiveness of the modified application schedule. However, animals of all experimental groups developed a broad spectrum of tumors in organs other than the skin and histological analysis of these neoplasms clearly reveals new properties of TPA. Based on detailed post-mortem examinations there are good reasons for believing that topically applied TPA has the ability to promote diaplacentally induced tumor germs in various organs. To that end, after local absorption the TPA must have reached the lymphatics and blood vessels and subsequently been distributed throughout the body. Besides the classical target organ, the skin, the liver was involved in tumor formation. These two organs were most susceptible to the combined treatment with both initiator and promoter although initiation alone at the same dose level was sufficient to produce tumors. Moreover, both DMBA/TPA- and urethane/TPA-combinations significantly stimulated tumor growth in organs which tend to show spontaneous tumors. A comparison with respect to the efficiency of the combination carcinogen/cocarcinogen and carcinogen alone (a- and b-groups) revealed differences in skin and liver but not in lung. The results of the procedure on the extent of tumor activation are impressive. There is an increased number of animals with multiple tumors and a considerably broadened spectrum of organs involved in tumor formation.

It can be suggested that in many cases initiation alone is not sufficient for active tumor expression during the normal life span. Apparently, however TPA promotion can shift a whole set of pre-programmed events to an earlier phase of life by activating initiated tumor cells ready to be promoted. Moreover, it is conceivable that the function of TPA can be taken over by other promoters (UV-light, hyperplasiogenic agents in the food and other environmental poisons). This fact, together with the assumption that prenatal initiation is by no means a rare event, indicates an increased risk also to the human organism.

It has been shown that in organs showing a distinct spontaneous tumor rate a much higher yield of tumors can be obtained by either combined treatment with an initiator and promoter or with an initiator alone. Based on this observation, a revision of the data concerning the influence of exogenous and endogenous factors in tumor induction is mandatory and urgently needed (see also Miller, 1973, 1977). The frequently encountered estimate of a ratio of about 90:10 is not soundly based since the possibility of combined genetic and exogenous damage must be taken into account. Obviously, for persons in which – due to a distinct genetical predisposition – tumors of defined localization can be produced by initiation/promotion or by initiation alone at a rate greater than the spontaneous tumor rate, the permissible limits for exogenous damage should be lower when compared with a control group which lacks this genetic handicap.

Among the numerous tumors observed those of the nervous system and the urogenital tract merit special interest. The capacity of diaplacentally administered nitroso compounds to produce tumors in these organs in the rat has been described by many authors in the last years (Druckrey, 1973; Magee, 1975; see also a review by Ivankovic, 1975). The occurrence of neurilemmonas

and meningeal sarcomas in the mouse also reveals this species to be prenatally susceptible in the same organ. It may be assumed that it is not the substance specifity but rather an organ predisposition which plays a role in the formation of the tumors. The influence of TPA on the kidney, vagina, uterus, and the ovaries – especially in the groups with combined DMBA/TPA treatment – suggests the speculation, that apart from a general stimulating capacity TPA may have additional hormone-like (oestrogen-like) properties. The effects of the 2-stage experiment on the vaginal wall are of special interest with regard to the findings of Herbst et al. (1971, 1975), since the tumors observed in NMRI mice resembled the alterations which were seen in humans after diaplacental administration of oestrogen in many aspects. Of course, the elucidation of possible additional properties of TPA (or the combination DMBA/TPA) needs further experimental studies. In this context it is an open question whether the initiation with DMBA represents the crucial event. The only pathological alteration we observed after DMBA initiation in the vaginal wall was an angioendothelioma. It is obvious that to eliminate possible side-effects of DMBA its dose should be reduced in further experiments.

It should be mentioned that the present experiment was unfavorably influenced by several weak points. We were not prepared for such important and frequent alterations in organs other than the skin within the investigation period of 52 weeks. Moreover, the comparatively low number of animals in the b-groups made the statistical calculations difficult. Similarly, the varying concentrations of DMBA and the application of the substance over several days during fetal life strongly impeded the determination of phases of special sensitivity[3].

The high dose of DMBA in subgroup 3 a did not lead to an increase in the number of tumors. However, a numerical augmentation of additional pathological alterations (mainly preneoplasias) in other organs could be observed. Whereas in subgroup 2 a only 9 of these alterations occurred, subgroup 3 a contained 19. This points to increased damage of the fetus. The high abortion rate of pregnant mother animals in group 3 has already been mentioned.

Our experiment on prenatal tumor production gives further insight into the mode of action of the tumor promoter TPA. We were able to show that upon postnatal treatment with TPA the prenatally initiated organism gives rise not only to the expected skin tumors but also to tumors in various organs. This wide-spread tumor-promoting property of TPA has not yet been described (see the review of Hecker, 1975). The underlying pathogenetic mechanism is now to be investigated. Based on findings which have been reported following administration of diethylstilboestrol, we tend to assume a hormone-like participation of TPA. In addition, supplementary information with regard to the action spectrum of TPA increases our present knowledge of prenatal carcinogenesis. As mentioned by Goerttler (1957), information available on possible damage to the prenatal organism during the organogenic embryonic period is much deeper

[3] In a current experiment we have tried to avoid these experimental deficiencies and to focus mainly on the problem of dosage and time of application of the initiator. First results indicate the presence of tumors in the vaginal wall and point to the extremely high sensibility of this organ when exposed to noxious effects during the late fetal period

than on the following so-called fetal developmental phase. However, on the other hand, we are still very ignorant with respect to possible noxious effects during the last third of fetal development. This situation is much improved compared with that of a decade ago (see review of Tomatis and Mohr, 1973). In 1965, Di Paolo and Kotin published a fundamental paper on the borderland between oncogenesis and teratogenesis which effectively stimulated the interest in this field. However, investigations on diaplacental carcinogenesis from several laboratories were centered mainly on nitroso compounds as carcinogens and on the nervous system as a target organ (Druckrey et al., 1968; Wechsler, 1972; Mennel and Zülch, 1972; Vesselinovitch, 1973). However, borderline cases between oncology and teratology have shown that numerous relationships and possibilites of damage can exist which go beyond the nervous system (Mulvihil, Miller and Fraumeni, 1977). We were able to demonstrate (Goerttler et al., 1971) that aflatoxin B1 is taken up diaplacentally and leads to an initial depression of DNA synthesis mainly in the liver followed by a long-lasting stimulation. Preliminary observations after application of aflatoxin B1 show tumor formation in many organs, including the brain. In 1968, Goerttler proposed that in addition to clearly defined teratogenetic determination periods (Schwalbe, 1906) oncogenetic determination periods should be taken into consideration during fetal life. According to Balmain et al. (1977), the electrophoretic protein pattern of mouse epidermis shows remarkable changes during the late fetal period. It would be of interest to know if these findings can be correlated with a varying sensibility to carcinogenic influence. The modified 2-stage Berenblum/Mottram experiment as described in this paper allows the study of problems on diaplacental carcinogenesis in a comparatively short time and gives results which are also relevant for human medicine.

References

Balmain, A., Loehren, D., Fischer, J., Alonso, A.: Protein synthesis during fetal development of mouse epidermis. I. The appearance of "histidine-rich protein". Develop. Biol. (1977) (in press)

Di Paolo, J., Kotin, P.: Teratogenesis-oncogenesis. A study of possible relationships. Arch. Path. **81**, 3–23 (1966)

Druckrey, H.: Chemical studies and actions in transplacental carcinogenesis and teratogenesis. In: Tomatis, L., Mohr, U. (eds.): Transplacental Carcinogenesis. IARC Scientific Publications, No. 4. Lyon: International Agency of Research on Cancer 1973

Druckrey, H., Ivankovic, S., Preussmann, R.T., Landschütz, C., Steker, J., Brunner, U., Schagen, B.: Transplacentar induction of neurogenic malignomas by 1,2-diethylhydrazine, azo-, and azoxyethane in rats. Experientia (Basel), **24**, 561–562 (1968)

Dunn, O.J.: Multiple comparisons using rank sums. Technometrics **6**, 241–252 (1964)

Gabriel, K.R.: Simultaneous test procedures for multiple comparisons on categorical data. J. Am. Stat. Ass. **61**, 1081–1096 (1966)

Goerttler, K.: Über terminologische und begriffliche Fragen der Pathologie der Pränatalzeit. Virchows Arch. path. Anat. **330**, 35–84 (1957)

Goerttler, K.: Experimentell-teratologische Aspekte zur Onkologie. In: H. Lettré und G. Wagner: Aktuelle Probleme aus dem Gebiet der Cancerologie II. pp. 34–41. Berlin-Heidelberg-New York: Springer 1968

Goerttler, K., Arnold, H.P., Michalk, D.: Über carcinogen-induzierte diaplacentare Wirkungen bei Ratten, Histologische Befunde und Kinetik des DNS-Stoffwechsels nach Gabe von Aflatoxin B_1, Methylazoxymethanol und Äthylnitrosoharnstoff. Z. Krebsforsch. **74**, 396–411 (1970)

Goerttler, K., Loehrke, H.: Transmaternal variation of the Berenblum experiment with NMRI-mice. Tumor initiation with DMBA via mothers milk followed by promotion with the phorbol ester TPA. Virchows Arch. A Path. Anat. and Histol. **370**, 97–102 (1976a)

Goerttler, K., Loehrke, H.: Diaplacental carcinogenesis: Initiation with the carcinogen dimethylbenzanthracene (DMBA) and urethane during fetal life and postnatal promotion with the phorbol ester TPA in a modified 2-stage Berenblum/Mottram experiment. Virchows Arch. A Path. Anat. and Histol. **372**, 29–38 (1976)

Hecker, E.: Cocarcinogens and Cocarcinogenesis (with a note on synergistic processes in carcinogenesis. In: E. Grundmann: Hdb. Allg. Path. Vol. 6/6 Geschwülste-Tumors II. Virale und Chemische Carcinogenese-Viral and Chemical Carcinogenesis, pp. 651–676, Berlin-Heidelberg-New York: Springer 1975

Herbst, A., Poscanzer, D.C., Robboy, S.J.: Prenatal exposure to stilbestrol: A prospective comparison of exposed female offspring with unexposed controls. New Engl. J. Med. **292**, 334–339 (1975)

Herbst, A., Ulfelder, L., Poscanzer, D.C.: Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. New Engl. J. Med. **284**, 878–881 (1971)

Ivankovic, S.: Pränatale Carcinogenese. In: E. Grundmann (ed.): Handbuch d. Allg. Path. Vol. 6/7 Geschwülste – Tumors III. Modelle experimenteller Carcinogenese. Models of experimental Carcinogenesis. pp. 941–1002, Berlin-Heidelberg-New York: Springer 1975

Kruskal, W.H., Wallis, W.A.: Use of ranks in one-criterion variance analysis. J. Am. Stat. Ass. **47**, 583–621 (1952)

Magee, P.N.: Transplacental carcinogenesis. Proc. Roy. Soc. Med. **68**, 655–657 (1975)

Mennel, H.D., Zülch, K.J.: Zur Morphologie transplacentar erzeugter neurogener Tumoren beim Goldhamster. Acta neuropath. (Berlin) **21**, 140–153 (1972)

Miller, R.W.: Prenatal origins of cancer in man: Epidemiological evidence. In: Tomatis, L., Mohr, U. (eds.): Transplacental Carcinogenesis. IARC Scientific Publications, No. 4, pp. 175–180. Lyon: International Agency für Research of Cancer 1973

Miller, R.W.: Relationship between human teratogens and carcinogens. J. Natl. Cancer Inst. **58**, 471–474 (1977)

Mulvihill, J.J., Miller, R.W., Fraumeni, J.F. jr. (eds.): Genetic of human cancer. New York: Raven 1977

Schwalbe, E.: Die Morphologie der Mißbildung des Menschen und der Tiere. 1. Teil. Allgemeine Mißbildungslehre (Teratologie). Fischer: Jena 1906

Tomatis, L., Mohr, U.) eds.): Transplacental carcinogenesis. IARC Scientific Publications No. 4. Lyon: International Agency for Research on Cancer 1973

Vesselinovitch, S.D.: Comparative studies on perinatal carcinogenesis. In: Tomatis, L., Mohr, U. (eds.): Transplacental Carcinogenesis. IARC Scientific Publications No. 4, pp. 14–22. Lyon: International Agency for Research on Cancer 1973

Wechsler, W.: Teratogenic and oncogenic effects of ethylnitrosourea in the developing nervous system of rats. In: Cavanagh (ed.), pp. 133–139. London: Ciba Symposium 1972

Received August 1, 1977