TRANSPLACENTAL ACTION OF 4-DIMETHYLAMINOAZOBENZENE AND DIETHYLAMINOAZOBENZENE ON ORGAN CULTIVATION OF MOUSE EMBRYONIC KIDNEY TISSUE

N. I. Golub'

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The transplacental action of 4-dimethylaminoazobenzene (DAB) and diethylaminoazobenzene (DEAB) was studied in vitro. The compounds were injected into female BALB/c mice in the last week of pregnancy in a total dose of 96-120 mg. Kidneys of embryos taken from the females were explanted in organ cultures until 25 days. The transplacental action of DEAB and DAB was manifested as higher survival of the experimental cultures compared with the controls and increased hyperplastic changes in the epithelium, which were more marked in the experiment with the carcinogen. These changes include diffuse and focal proliferation and layers of atypical epithelium.

Earlier work demonstrated the transplacental action of certain carcinogenic agents [8, 11-13]. Further research in this direction began comparatively recently [4, 5, 9, 14].

Previous investigations in the author's laboratory showed that organ cultures of kidneys are also suitable objects for studying the transplacental action of carcinogenic compounds [3, 6, 7].

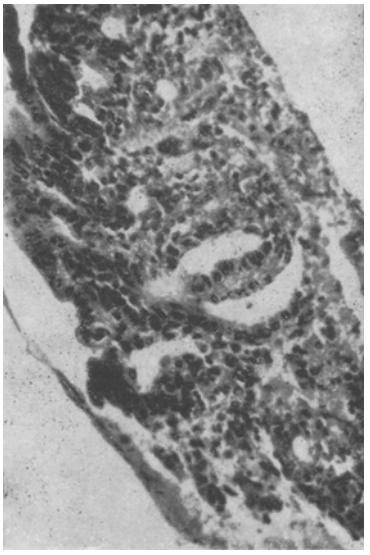

Fig. 1. Focal proliferation of hyperplastic, hyperchromic tubular epithelium. Here and in Figs. 2 and 3: DAB, 18th day of cultivation. Hematoxylin-eosin, 140 ×.

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TABLE 1. Survival Rate and Hyperplastic Proliferation of Epithelium in Organ Cultures of Mouse Embryonic Kidney Tissue Exposed to the Transplacental Action of DAB and DEAB

Duration of experiment (in days)	Control						DEAB							DAB						
	number of explants		hyperplastic proliferation of tubular epithelium				duration of experiment (in days)	number of explants		hyperplastic proliferation of tubular epithelium				duration of experiment (in days)	number of explants		hyperplastic proliferation of tubular epithelium			
	total	alive	1	2	3	4		total	alive	1	2	3	4		total	alive	1	2	3	4
5	40	40	—	—	—	—	5	35	34	2	2	—	—	5	64	62	8	8	5	10
7	68	60	1	—	—	—	7	51	51	3	2	—	1	7	69	69	7	6	2	5
10	42	22	—	—	—	—	10	50	44	3	5	—	—	10	68	58	2	—	—	7
15	45	4	—	—	—	—	15	50	43	2	1	—	—	15	62	57	—	4	—	—
18	32	2	—	—	—	—	18	55	46	1	—	1	—	18	75	56	—	1	2	6
21	35	—	—	—	—	—	21	58	42	1	1	—	—	21	59	37	—	—	—	3
25	—	—	—	—	—	—	25	46	32	—	—	—	—	25	35	16	—	—	—	—
Total ...	262	128	1	—	—	—		345	292	12	11	1	1		434	355	17	19	9	31
%	48		0,8						84	4,1	3,7	0,3	0,3		82		4,8	5,3	2,5	8,7

Legend: 1) Projections; 2) diffuse hyperplasia of tubular epithelium; 3) epithelial layers; 4) focal proliferation of tubular epithelium.

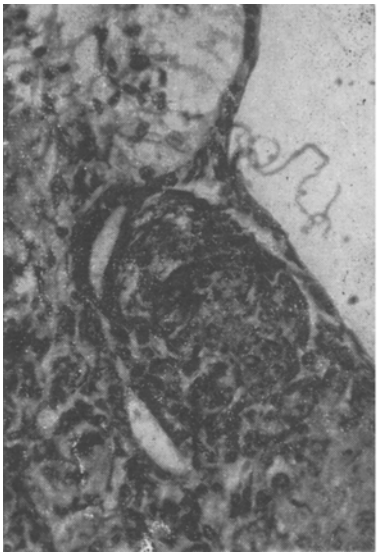

Fig. 2. Adenomatous growths formed of hyperplastic epithelial cells.

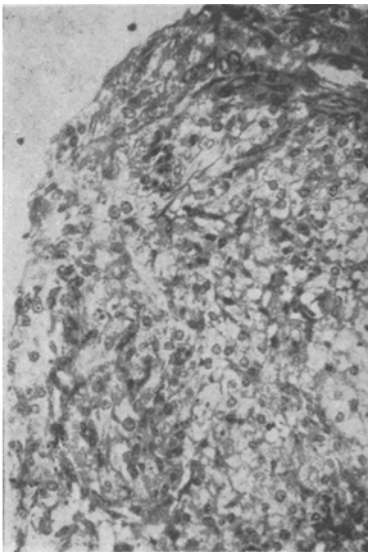

Fig. 3. Continuous layer of atypical epithelium occupying a large part of the explant.

In the present investigation the sensitivity of mouse embryonic kidney tissue to 4-dimethylaminoazobenzene (DAB) and to its noncarcinogenic analog diethylaminoazobenzene (DEAB) was studied. DAB is known to be a powerful carcinogenic agent [2].

EXPERIMENTAL METHOD

Female BALB/c mice in the last week of pregnancy received a subcutaneous injection of DAB and DEAB in a dose of 24 mg in 0.1 ml sunflower oil, repeated 4-5 times, i.e., the total dose received by the animal was 96-120 mg of the compound. The technique of cultivation was that suggested by Chen [10] and modified by Adil'gireeva [1] and Kolesnichenko [4]. Altogether 1,031 explants were studied.

EXPERIMENTAL RESULTS

The results are summarized in Tables 1 and 2. As Table 1 shows, investigation of explants subjected to the action of DAB and DEAB revealed better survival of the experimental cultures than of the controls. Altogether 48% of the control explants survived compared with 84% in the experiment with DEAB and 82% of explants in the experiment with DAB.

A study of the control cultures on the 4th day showed that in most explants the kidney structure as a whole remained intact. After 9-12 days the cultures consisted mainly of convoluted tubules. Starting from the 12th day, degenerative changes became intensified and by the 15th-18th day the explants were virtually all dead.

In the control explants on the 7th day of cultivation a unique type of projection was found. It consisted of pale epithelial cells and appeared to be a tubule growing beyond the limits of the fragment.

A different picture was found during the investigation of the experimental cultures, in which a series of hyperplastic growths of the epithelium was found throughout the period of cultivation, their number being considerably greater than the number of the corresponding structures in the control (Table 2).

The differences between the morphological changes found in the experimental and control series were not only quantitative, but also qualitative. For example, in explants exposed to the action of the aminoazo-compounds various diffuse and focal hyperplastic growths of the epithelium and the formation of continuous layers of atypical epithelium were observed. Changes of this type (except the structural projections) were never observed in the control series (Table 2). Besides diffuse proliferation, which occurred after the transplacental action of both compounds, foci of proliferation also were found (Fig. 1), and these

TABLE 2. Final Results of Action of DEAB and DAB on Kidney Organ Cultures

Distribution of hyperplastic proliferation of epithelium of the types given below	Control	DEAB	DAB
Projections	0,8%	4,1% $P_{C-1}<0,05$	4,8% $P_{C-2}<0,01$ $P_{1-2}>0,05$
Diffuse hyperplasia of tubular epithelium	—	3,7% $P_{C-1}<0,05$	5,3% $P_{C-2}<0,01$ $P_{1-2}>0,05$
Epithelial layer	—	0,3% $P_{C-1}>0,05$	2,5% $P_{C-2}>0,05$ $P_{1-2}<0,05$
Focal proliferation of tubular epithelium	—	0,3% $P_{C-1}>0,05$	8,7% $P_{C-2}<0,01$ $P_{1-2}<0,01$

Legend: P – criterion of significance determined from Student–Fisher table; P_{C-1} – statistical significance of difference between action of DEAB and control; P_{C-2} – statistical significance of difference between action of DAB and control; P_{1-2} – statistical significance of difference between action of DEAB and DAB.

foci were more numerous in the experiment with the carcinogen (Table 2). In individual cases the foci of proliferation resembled adenomatous growths (Fig. 2). Finally, after exposure to DAB epithelial layers consisting of atypical epithelial cells were found (Fig. 3).

Hence, organ cultivation of the kidneys of embryos taken from mice exposed during pregnancy to the action of DEAB and DAB showed that these compounds improve the changes of survival of the kidney cultures. The higher survival rate during cultivation was evidently due to the increased hyperplastic proliferation of the epithelium. Among the growths observed following exposure to the carcinogen (and much less frequently, to its noncarcinogenic analog) structures not found in the control were seen. These include diffuse and focal proliferation and layers of atypical epithelium.

The results show that there is no direct correlation between the changes observed in the kidney tissue and the carcinogenic action of these two compounds. For example, DAB is a powerful hepatic carcinogen and induces tumors predominantly in rats. In the present investigation it induced hyperplastic growths of the renal epithelium in mice. The noncarcinogenic compound DEAB induced the same growths of the epithelium, although in smaller numbers. It must not be forgotten that N-oxy metabolites of aminoazo-compounds may be formed in the body of the pregnant animal, and that these may be more active than the original substance. Finally, it is possible that the cell-called noncarcinogenic analogs, which are similar in structure to the carcinogen, may under certain conditions exhibit a weak carcinogenic action [15]. All these considerations justify further research.

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