TRANSPLACENTAL ACTION OF 3,3'-DICHLOROBENZIDINE AND ORTHOTOLIDINE ON ORGAN CULTURES OF EMBRYONIC MOUSE KIDNEY TISSUE

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The effect of 3,3'-dichlorobenzidine and orthotolidine on pregnant mice was to produce better survival of experimental organ cultures of mouse embryonic kidney than in controls and to produce hyperplastic changes in the epithelium not observed in the controls.

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Carcinogenic effects on the organism during embryogenesis are a problem of great interest. The possibility of transplacental carcinogenesis has been demonstrated experimentally [5]. Further work in this direction began comparatively recently [2, 6, 7]. Kolesnichenko [2], for instance, showed that adenomas may develop in organ cultures of embryonic lung tissue from mice receiving urethane during the last days of pregnancy. The first adenoma was found on the 4th day of explanation. In another investigation, when organ cultures of mouse lung tissue were injected to the transplacental action of nitrosamines, foci of hyperplasia resembling adenomatosis were observed.

Sensitivity of mouse embryonic kidney tissue to 3,3'-dichlorobenzidine (DCB) and to orthotolidine (OT) acting by the transplacental route in the prenatal period, followed by organ culture of the embryonic kidney tissue, was studied in this investigation. The marked carcinogenic activity of DCB was first discovered by Pliss [3] in 1959. OT also possesses carcinogenic activity [4].

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c mice in accordance with the following scheme. Males and females were mated, and from the following day and throughout the period of pregnancy the females received DCB or OT in a dose of 2 mg in 0.1 ml sunflower oil by daily subcutaneous injection. The pregnant mice were sacrificed on the 19th-20th day and the embryonic kidneys used for organ culture by the method suggested by Chen [8] and modified by Adil'gireeva [1] and Kolesnichenko [2]. The duration of cultivation was 18-20 days. Explants were investigated on the 3rd-4th, 6th-7th, 14th-15th, and 18th-20th days. Altogether 92 experimental and 55 control explants were studied.

EXPERIMENTAL RESULTS

In most control explants on the 3rd-4th day of cultivation the kidney structure was largely intact. In some explants, however, the first signs of degeneration appeared, with detritus visible in individual glomeruli and in the space between Bowman's capsule and the glomerulus itself, and with a homogeneous, well stained protein material in the lumen of the tubules. By the 7th day of cultivation, considerable disturbances of the medulla and part of the cortex could be seen. Glomeruli had almost completely disappeared from the cortex. Death of the glomeruli took place in two ways: either by disintegration of the glomerulus and capsule, resulting in formation of clearly visible chromatin masses, or disintegration of the glomerulus through lysis of its cells, in which case only the empty capsule remained. Large numbers

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TABLE 1. Survival of Kidney Explants from BALB/c Mice at Different Times of Cultivation and Morphological Changes in Them

Compound tested	Duration of cultivation (in days)	Number of explants	Survival of explants		Morphological changes	
			abs.	%	abs.	%
Control	3—4	13	13	100	—	—
	6—7	12	12	100	—	—
	14—15	23	0	0	—	—
	18—20	7	0	0	—	—
	Total	55				
Dichlorobenzidine	3—4	3	3	100	—	—
	6—7	6	6	100	2	33,3
	14—15	48	40	83	15	31,3
	18—20	9	5	55	3	33,3
	Total . . .	66			20	33,3
Orthotolidine	3—4	6	6	100	4	66,6
	6—7	8	8	100	2	25,0
	14—15	12	8	66	4	33,3
	Total	26	—		10	38,4

*No such morphological changes were observed in the control cultures.

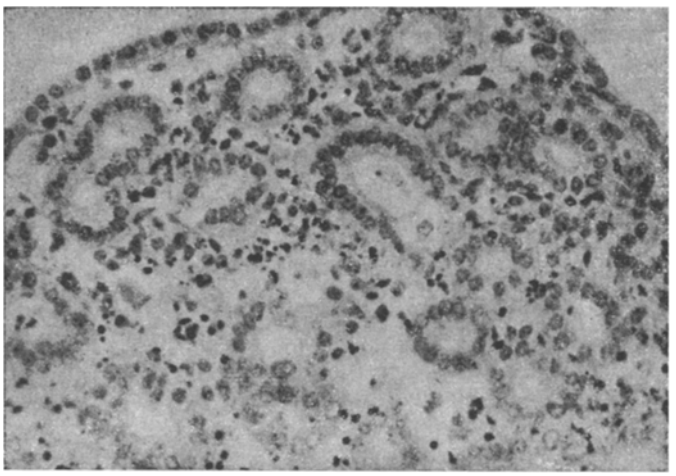

Fig. 1. Hyperplastic foci of epithelium after transplacental action of DCB. Hematoxylin-eosin, 140×.

of connective-tissue cells appeared in the medulla, while a few descending ducts and occasional collecting tubules remained. If cultivation continued, the explants practically all had died by the 14th-15th day. Almost complete degeneration was observed in the medulla. Sometimes occasional descending ducts were found, while in the cortex only solitary convoluted tubules and epithelial cells remained. Necrosis was complete by the 18th day of cultivation.

A different picture was observed when experimental explants exposed to the transplacental action of the carcinogenic compounds were investigated. Although they were indistinguishable from the controls on the 3rd-4th day of cultivation, on subsequent days differences between them and the control explants began to appear and to increase sharply in degree. By the 6th-7th day of cultivation, in the case of exposure to

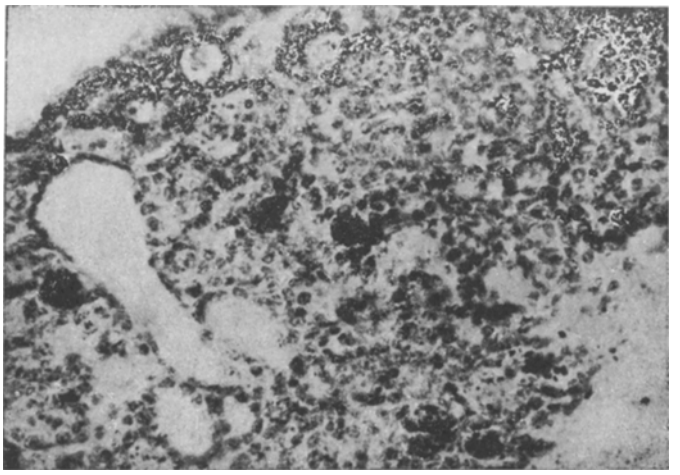

Fig. 2. Hyperchromic glomeruli after transplacental action of DCB. Hematoxylin-eosin, 140×.

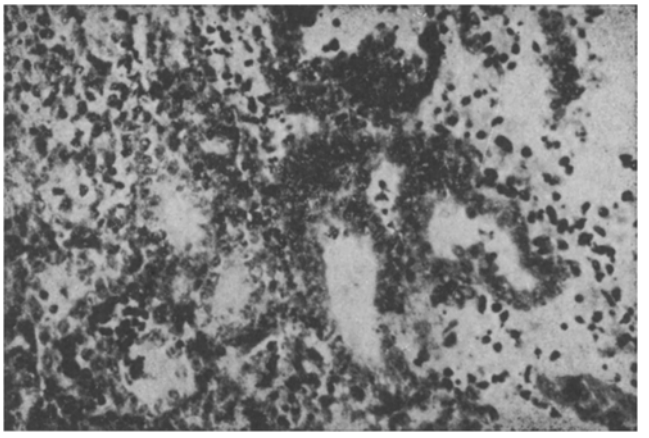

Fig. 3. Hyperplastic foci of tubular epithelium after transplacental action of OT. Hematoxylin-eosin, 140×.

DCB, almost complete preservation of the kidney structure resembling in its morphology that of the control cultures was observed in 33% of explants on the 3rd day of cultivation. In the control series, on the other hand, no explants with this morphological picture were observed at this period. The most marked differences were seen on the 14th-15th day, when virtually all explants in the control series had died, while in the experimental series 83% survived. On the 18th-20th day, complete necrosis was observed in the control, whereas in the experimental series 55% of explants were still viable. The same pattern was observed in the experiments with OT (Table 1).

In the experiments with both DCB and OT, definite morphological changes were observed which were not found in the control cultures. In the experiments with DCB, for instance, they were found on the 6th-7th day and reached a maximum by the 14th-15th day. Their total number varied around 33%. In the experiments with OT, on the other hand, they appeared on the 3rd-4th day and their total number was 38% (Table 1).

The morphological characteristics of these changes will be discussed in more detail. In the experiments with DCB, outgrowths covered by a clearly visible capsule, consisting of cubical epithelial cells (Fig. 1), were found at the periphery of the explant. Inside these outgrowths the normal kidney structure was preserved. However, enlargement of the glomeruli and the cells of the tubules and widening of the lumen of the tubules were observed. In some cases hyperchromic glomeruli appeared (Fig. 2). After the action of OT, in some explants hyperplasia of the tubular epithelial cells was observed, resulting in the

formation of well defined foci of hyperplasia (Fig. 3). In some explants the changes observed were similar to those found during the action of DCB.

Organ culture of embryonic kidneys taken from mice receiving DCB and OT during pregnancy thus confirmed their transplacental effects. First, these compounds improve the survival rate of experimental cultures compared with the controls. Second, both DCB and OT cause the appearance of morphological changes leading to hyerplasia of epithelial structures.

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