that it is much less intensive than in mice; there is thus a need to study ways and means of intensifying migration of HSC by additional forms of stimulation or by bone marrow autografts.

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GROWTH-STIMULATING ACTION OF SOME NITROSO COMPOUNDS ON ORGAN CULTURES OF MOUSE AND RAT EMBRYONIC LIVER

T. S. Kolesnichenko, N. V. Popova, and L. M. Shabad

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The transplacental and direct action of nitrosomethylurea on organ cultures of the liver from 18-20-day CBA and C57BL mouse embryos and of diethylnitrosamine on liver cultures from noninbred rat embryos was studied. The nitroso compounds accelerated adaptation of the explants, increased the viability of the cultures compared with normal, and led to hyperplastic proliferation of the small basophilic cells whose survival rate under both normal and experimental conditions was higher than that of ordinary embryonic hepatocytes. The growth-stimulating effect depended on the object (species, line of animals) and the factor to which it was exposed (carcinogen, mode of administration).

KEY WORDS: nitroso compounds; transplacental carcinogenesis; organ cultures; embryonic liver.

Among the many carcinogens nitroso compounds (NC) are distinguished by their high biological activity and the broad spectrum of the tumors they induce, when these substances are administered in different ways, including by the transplacental route [11, 14]. The possibility of endogenous synthesis of carcinogenic NC from noncarcinogenic precursors, the wide use of NC in various branches of the economy, and their spread in the external environment create conditions for human contact with them [3]. A high sensitivity of the embryo to chemical agents, and to NC in particular, makes the study of various aspects of their action on embryonic tissues with a view to detecting pathological changes one of the utmost urgency.

Previous investigations showed that one possible way of studying chemical carcinogenesis, especially the transplacental kind, is simulation of this process by the organotypical culture of embryonic target tissues [4, 11]. By this means a systematic study could be made of the mechanisms of action of chemical carcinogens on embryonic tissues in animals and man. Species and interlinear differences in the sensitivity of embryonic lungs to the toxic and growth-stimulating action of some NC were discovered previously [5].

The object of this investigation was to compare organ cultures of mouse and rat embryonic liver under normal conditions and following transplacental and direct exposure to nitrosomethylurea (NMU) and diethylnitrosamine (DENA).

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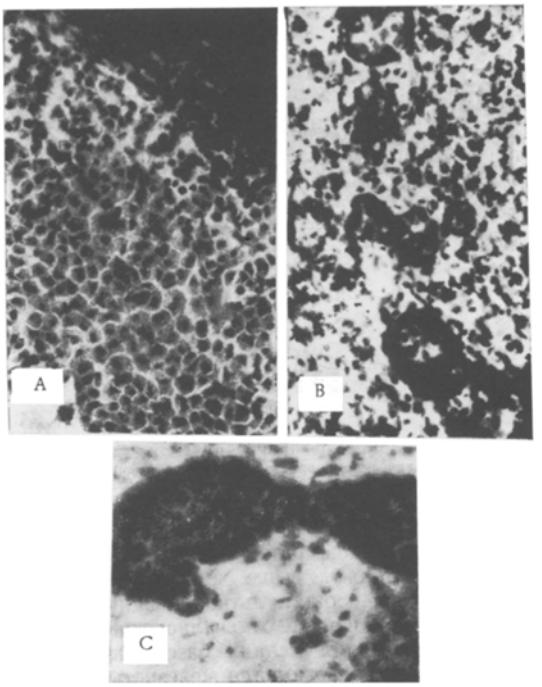

Fig. 1. Normal embryonic mouse liver (125 ×). A) Zone of growth of hepatocytes around explant (CBA mouse, 4th day in culture); B) glandular structures composed of small basophilic cells (C57BL mouse, 30th day of culture); C) solid sheet of small basophilic cells (CBA mouse, 23rd day in culture).

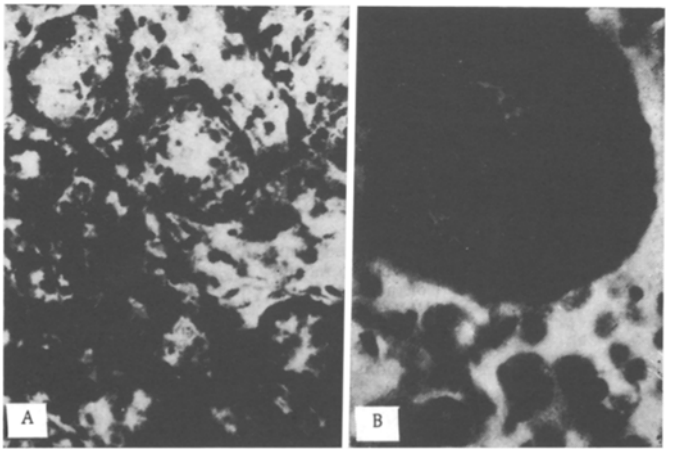

Fig. 2. Mouse embryonic liver, action of NMU. A) Hyperplastic glandular growth of small basophilic cells (C57BL mouse, NMU 0.05 mg/ml medium, 30th day of culture, 300 ×); B) solid sheet of small basophilic cells (CBA mouse, NMU 10 mg/kg, 23rd day of culture, 300 ×).

EXPERIMENTAL METHOD

Experiments were carried out on organ cultures of embryonic liver from 18-20-day embryos of CBA and C57BL mice, and noninbred rats. To study the direct action of NMU on embryonic mouse liver and of DENA on embryonic rat liver the carcinogens were added to the nutrient medium of the cultures in a concentration of 0.05 mg/ml on the first day of the experiment, and subsequently at each change of medium, i.e., every 3-4 days.

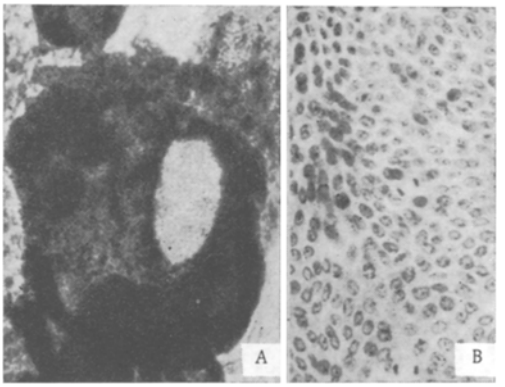

Fig. 3. Rat embryonic liver, action of DENA (2 mg/kg, 34th day of culture). A) Hyperplastic growth of small basophilic cells forming dense nodule (125×); B) solid sheet of small basophilic cells, showing mitoses (300×).

Culture in the presence of the carcinogens continued until the 15th day, and in their absence for a further 30-37 days. To study the transplacental action of the carcinogens they were administered to female animals during the last third of pregnancy: NMU to mice (three subcutaneous injections, each of 10 mg/kg, in 0.2 ml Hanks's solution), DENA to rats (three subcutaneous injections, each of 2 mg/kg, in 0.3 ml Hanks's solution). On the 18th-20th day of pregnancy the experimental females were killed and the livers of the embryos were explanted into organ culture. Corresponding cultures of normal embryonic liver from intact animals served as the control. The explants were examined at intervals of 3-4 days in total preparations stained with hematoxylin. The cultures were grown by E. A. Luriya's method [8] in the modification adopted in the writers' laboratory [6].

EXPERIMENTAL RESULTS

During organotypical culture of the liver from intact mouse and rat embryos similar features were observed. On the 2nd-3rd day the explants spread out over the surface of the filter. Migration of hematopoietic cells and macrophages outside the boundaries of the explant and the formation of a zone of growth around it from hepatocytes with the characteristic polygonal shape, and with a pale round nucleus containing one or two nucleoli, were observed (Fig. 1A). Meanwhile characteristic primary central necroses characteristic of organ cultures appeared. Products of cell destruction were actively phagocytosed by macrophages. On the subsequent days further spreading of the explants took place, with enlargement of the zone of growth of the hepatocytes; the central necrotic foci were replaced by epithelium. By the 13th-15th day the cultures consisted of sheets of epithelium, 1 to 5 layers thick, with no clear boundaries between the zone of growth and the explant tissue proper. Beneath the epithelium connective-tissue cells growing into pores of the filter and spreading over its lower surface as a network of small branched cells with irregularly shaped hyperchromic nuclei could be seen. Among the ordinary embryonic hepatocytes, which were the majority, foci of growth of small round or oval basophilic cells with a comparatively large nucleus, surrounded by a narrow rim of cytoplasm, were seen. Being tightly packed together, these cells formed solid structureless sheets or bands (Fig. 1C). Here and there cyst-like and glandular structures were seen (Fig. 1B). The morphology and character of growth of these cells pointed to their epithelial nature. The homogeneity of the cells in the foci of growth suggested that they were clonal in origin.

Intensive hematopoiesis in mouse embryonic liver cultures was observed until 16th-18th day, and in rat cultures until the 11th-14th day. Later it gradually diminished and ceased altogether by the 22nd-25th day, after which degenerative changes began to develop in the cultures. However, foci of proliferation of the small basophilic cells described above still remained completely viable under these circumstances.

Under the influence of the doses of NMU and DENA used, whether by transplacental or direct action in vitro, the explants adapted themselves more rapidly to the conditions of growth in vitro. The zone of growth of the hepatocytes developed more rapidly in them. The survival rate of the explants and the frequency of foci of proliferation of small basophilic cells were higher in the experimental than in the control groups, especially

TABLE 1. Transplacental and Direct Action of NMU and DENA on Mouse and Rat Embryonic Liver Organ Cultures

Species and line of animals	Experimental conditions	Number of explants studied	Survival rate of explants, %		Percentage of explants with foci of growth of small basophilic cells	
			mean for period of culture—from 3rd to 37th days	in late stages of culture—from 25th to 37th days	mean for period of culture—from 3rd to 37th days	in late stages of culture—from 25th to 37th days
CBA mice	Control	936	55,5	19,2—25,0	6,2	3,8—8,3
	NMU:					
	Transplacental	225	77,3	62,5—69,2	12,8	18,4
	Direct	312	83,5	72,5—92,2	13,1	32,5—40,0
C57BL mice	Control	146	65,5	42,8—46,1	21,8	34,3—53,8
	NMU:					
	Transplacental	172	77,7	72,0—76,7	46,5	80,0—96,4
	Direct	125	89,5	85,7—100,0	28,0	41,2—63,6
Noninbred rats	Control	446	75,0	53,3	9,4	7,3—16,7
	DENA:					
	Transplacental	179	80,0	86,7—98,1	60,0	83,3—100,0
	Direct	306	96,5	88,2—98,2	20,3	25,0—42,8

in the late stages of culture (Table 1). In the experimental series hyperplastic growths of small basophilic cells were observed. As the result, glandular growth (Fig. 2A), solid sheets of cells (Fig. 2B), polycystic structures and dense nodules (Fig. 3A), surrounded by solid sheets of these cells with numerous mitoses (Fig. 3B) appeared. The viability of these hyperplastic zones of proliferation was considerably higher than that of the embryonic hepatocytes. As Table 1 shows, both under normal conditions and under the influence of NC, definite species and interlinear differences in the survival rate and frequency of focal growths of small basophilic cells were observed. The latter proved to be most sensitive to the growth-stimulating action of NC. Similar results were obtained previously by the writers in experiments with orthoaminoazotoluene (OAAT) on embryonic liver organ cultures from CBA mice [6]. Administration of the same doses of OAAT to pregnant CBA mice led to the appearance of many liver tumors in their progeny [7]. It can be tentatively suggested that the changes found under the influence of NMU, DENA, and OAAT in the organ cultures were early manifestations of tumor growth and that the small basophilic cells which are being discussed play an essential role in hepatocarcinogenesis. Attention is drawn to the morphological similarity of these cells found in cultures with the so-called oval cells appearing in the liver of animals in the early stages of induced hepatocarcinogenesis [1, 2, 12].

NMU and DENA thus had a general growth-stimulating action on the cultures, increasing their viability, and in particular on the small basophilic cells, inducing their hyperplastic proliferation. The growth-stimulating effect depended both on the object and on the agent used. It was greatest in liver cultures of rats and CBA mice, which are susceptible to both spontaneous and induced liver tumors. In embryonic liver cultures of C57BL mice, which are resistant to spontaneous and induced liver tumors, the growth-stimulating effect was weaker. The writers made similar observations previously in experiments with NC and other carcinogens to study their action on embryonic target tissues of susceptible and resistant animals [10, 11].

Comparison of the action of NC on the cultures revealed no significant difference in the results as a result of the transplacental and direct action of NMU. In experiments with DENA, the growth-stimulating action on rat liver cultures, especially on the small basophilic cells, was more marked in the case of the transplacental action of the carcinogen. These results can be explained by differences in the mechanism of action of the NC used. Metabolic activation of nitrosamine, to which group DENA and many other carcinogens belong, is known to require the participation of the enzyme systems of the host. This is not essential, however, for the nitrosamides, which include NMU [13]. The higher growth-stimulating and carcinogenic effects of DENA in the case of transplacental action on cultures of the liver and other organs [5, 9-11] are evidence that metabolic activation of the carcinogens during transplacental carcinogenesis evidently takes place chiefly in the mother, and that these metabolites are sufficiently stable to reach the fetal tissues.

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CELLULAR REGULATION OF SECRETION OF MITOGENIC FACTOR BY HUMAN LYMPHOCYTES IN VITRO

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The principles governing production of mitogenic factor in human lymphocyte cultures stimulated by phytohemagglutinin (PHA) were studied. The mitogenic activity of the culture media were tested in the presence of antibodies against PHA. Irradiation of the lymphocytes sharply increased their ability to produce mitogenic factors. Removal of phagocytic cells by means of iron carbonyl also led to marked stimulation of production of the factor. Irradiations and removal of phagocytic cells were shown to stimulate production of mitogenic factor by different mechanisms.

KEY WORDS: lymphokines; mitogenic factor.

Mitogenic factor (MF) is produced by human and animal lymphocytes in response to nonspecific or antigenic stimulation and is detected by its ability to induce proliferation of lymphocytes in culture. It is difficult at present to decide what type of intercellular interaction in immunogenesis in vivo is mediated through the liberation of MF. Meanwhile a secondary nonspecific mitogenic signal is necessary for the response of B-cells to be induced by antigen [6]. It has recently been shown that mitogenic lymphokines in vitro induce formation of killer cells in response to soluble transplantation antigens and, in the absence of antigens, may induce polyclonal stimulation of precursor cells of T-killers [8, 9]. Antibodies against MF suppress the proliferative response in mixed lymphocyte cultures [7]. Obtaining highly active MF is an essential condition for the study of its properties and role in the development of immune reactions. It must be emphasized that in vitro MF is produced inconstantly, often with low activity [10].

The writers showed previously that secretion of MF is sharply increased when protein synthesis is inhibited in stimulated lymphocytes [3]. However, the heterogeneity of MF [4, 7] arouses misgivings that the factor obtained under these conditions may have an incomplete spectrum of biological activity.

The present writers have suggested that variability of MF production during stimulation of lymphocytes is attributable to the existence of certain mechanisms controlling its production, and the aim of the present investigation was to discover these mechanisms.

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