the fact that it was induced by o-aminoazotoluene, a carcinogen which differs chemically from BP and, second, by the independent progression of the features of the malignant tumors [8].

At the cell level, correlation was thus found between the intensity of BP metabolism by hepatocytes and its toxic action on them. It was also shown that even sharply dedifferentiated cells of malignant tumors of the liver can retain their ability to metabolize BP and to exhibit sensitivity to its toxic action.

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SENSITIVITY OF MOUSE, RAT, AND HUMAN EMBRYONIC LUNGS TO THE ACTION OF NITROSOMETHYLUREA IN ORGAN CULTURE

T. S. Kolesnichenko

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In response to the direct action of nitrosomethylurea (NMU) in a concentration of 0.05 mg/ml on organ cultures of embryonic lungs of strain A mice, Wistar rats, and man, a varied degree of degenerative changes and hyperplastic proliferation of the epithelium developed in the cultures. In the early stages of the experiment the toxic effect of the cultures predominated. Tissue of rat embryonic lungs was most sensitive to the toxic action of NMU, mouse lung tissue least sensitive. The frequency of hyperplastic proliferation, on the other hand, was highest in cultures of mouse lungs and lowest in cultures of rat lungs. During culture the sensitivity of the human and rodent embryonic lungs to the toxic action of NMU decreased when the substance was repeatedly added to the nutrient medium. Meanwhile an increase in the survival of the experimental cultures was observed compared with the intact control.

KEY WORDS: *nitrosomethylurea; organ culture; embryonic lungs.*

The comparative study of the sensitivity of the tissues of animals and man to the action of carcinogenic agents is of theoretical interest and may be of the utmost importance to the

Department of Carcinogenic Agents, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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assessment of the potential risk of chemical substances to man. The question of the relationship between the toxic and carcinogenic effects of chemicals is particularly interesting.

In investigations of this sort cell and tissue cultures are widely used [1]. The most promising in this respect, in the writer's view, are organ cultures, in which the characteristic organotypical structure and some of the functional properties of the explanted tissues are preserved. During the action of biologically active substances and, in particular, of carcinogens processes similar to those developing in vivo are observed in organ cultures [11, 12].

This paper describes the results of a comparative study of the sensitivity of organ cultures on mouse, rat, and human embryonic lung tissue to the direct action of nitrosomethylurea (NMU), a carcinogen belonging to the group of nitroso compounds, whose biological activity is associated with alkylation reactions [14]. These substances induce various tumors. They and their precursors are widespread in the environment; they are used in different branches of the national economy and in medicine. In this connection the study of the sensitivity of animal and human tissues to the action of these compounds is most interesting.

EXPERIMENTAL METHOD

Lungs from 18- to 20-day strain A mouse and Wistar rat embryos and the lungs of 20- to 22-week human fetuses were used. The technique of organ culture was described by the writer previously [4, 11]. The solution of NMU was prepared immediately before use and added to the nutrient medium in a concentration of 0.05 mg/ml on the first day of the experiment and whenever the medium was changed, i.e., at intervals of 2-3 days. After the 15th day of the experiment, culture continued without the carcinogen. The explants were investigated morphologically in serial sections stained with hematoxylin and eosin, on the 8th, 15th, and 28th days of the experiment. The corresponding intact organ cultures of normal mouse, rat, and human embryonic lung tissue served as the control (Table 1).

EXPERIMENTAL RESULTS

Morphogenesis of intact organ cultures of normal mouse, rat, and human embryonic lung tissue was described by the writer previously [4, 6, 8, 10, 11].

TABLE 1. Action of NMU (0.05 mg/ml medium) on Organ Culture of Mouse, Rat, and Human Embryonic Lung Tissue

Donor	Experiment and control	Duration of culture, days	Number of cultures tested				
			total	with degenerative changes		with hyperplastic changes	
				abs.	%	abs.	%
Mouse	Experiment	8	110	17	15.5	44	40.0
		15	73	12	16.5	34	46.6
		28	68	15	22.1	24	35.4
	Control	8	120	17	14.2	—	—
		15	72	8	11.0	—	—
		28	78	53	67.9	—	—
Rat	Experiment	8	61	54	88.6	7	11.4
		15	85	61	71.8	22	25.8
		28	97	70	72.1	19	19.6
	Control	8	80	8	10.0	—	—
		15	85	43	51.1	—	—
		28	110	99	90.9	—	—
Man	Experiment	8	52	44	84.6	4	7.7
		15	55	25	44.5	15	27.3
		28	40	25	62.5	15	37.5
	Control	8	149	85	57.0	—	—
		15	48	22	45.9	—	—
		28	88	77	87.5	—	—

Treatment with NMU induced several characteristic changes in the morphology and survival rate of the organ cultures of the human and rodent embryonic lungs. For instance, by the 8th day of culture, degenerative changes in the cells were substantially increased in the experimental explants of rats and human embryonic lungs compared with the control.

In cultures of rat lungs the number of degeneratively changed cells reached 88.6% compared with 10.0% in the control ($P < 0.001$), and in the human lung cultures it was 84.6% compared with 57.0% in the control ($P < 0.01$). The same exposure to NMU had practically no toxic effect on the cultures of embryonic mouse lungs (Table 1).

Later, despite continuing exposure to NMU, no increase in the toxic effect was observed in the cultures. Conversely, by the 15th day the number of cells with degenerative changes was substantially less than on the 8th day of the experiment: in the experimental cultures of rat lungs by 17% ($P < 0.01$) and in the cultures of human lungs by 40% ($P < 0.001$). Subsequent culture without exposure to the carcinogen did not affect the frequency of degenerative changes in the cultures of rat lungs. They were a little more frequent in explants of mouse lungs and considerably increased in explants of human lungs. However, it will be clear from Table 1 that degenerative changes were found more often in the experimental than in the control cultures. For instance, in the experimental cultures of mouse lungs they amounted to 22.1% compared with 67.9% in the control ($P < 0.001$), in rats 72.1% compared with 90.9% in the control ($P < 0.01$), and in man 62.5% compared with 87.52% in the control ($P < 0.01$).

During repeated exposure to NMU the sensitivity of the embryonic lung tissue to its toxic action falls and the survival rate of the explanted tissue in the late stages of culture rises.

Besides the processes described above, hyperplastic changes in the epithelium also developed in the experimental cultures. Characteristic diffuse and focal areas of proliferation of the alveolar and bronchial epithelium similar to those arising through the transplacental action of NMU on the embryonic lungs in utero, described by the writer previously [10], were found in the mouse lungs. Predominantly diffuse-focal areas of hyperplastic proliferation of bronchial epithelium developed in the rat lungs, but their intensity was much lower than that observed after transplacental exposure to NMU [6]. Hyperplastic changes in the epithelium induced by the direct action of NMU in vitro in the human embryonic lungs were less intensive than in the rodents. Mainly they were represented by small local foci of proliferation of the bronchiolar epithelium, partly or completely occluding the lumen of the bronchioles. In a few cases mixed hyperplastic zones of proliferation of the epithelium and interstitial connective tissue were observed. The morphogenesis of the changes arising in organ cultures of human embryonic lungs under the influence of NMU were described by the writer previously [7].

Hyperplastic zones of proliferation of the epithelium developed most frequently in experimental cultures of strain A mouse embryonic lungs, which are very sensitive to the pulmonotropic carcinogenic action of various carcinogens including NMU [3, 5], but are resistant to the toxic action of NMU in the concentration used. However, it should be noted that gross degenerative changes in the cells, revealed by morphological investigations, are an extreme manifestation of the harmful effect of carcinogenic substances [1]. In the present case there were probably finer injuries to the cells that were optimal for the subsequent stages of tumor growth and, in particular, for the development of hyperplastic pretumor proliferation of the epithelium. In the experimental cultures of rat embryonic lungs foci of hyperplastic proliferation of the epithelium were much less frequent than in mice, possibly because of the powerful toxic action of NMU, which caused death of most explants in the early period of the experiment. The possibility cannot be ruled out that the result of the experiments in vitro reflect the lower sensitivity of rat lungs than of mouse lungs to the pulmonotropic carcinogenic action of nitroso compounds [3]. Human embryonic lung tissue occupied an intermediate position between mouse and rat embryonic lung tissues in its sensitivity to the toxic action of NMU in concentrations used and in the frequency of development of hyperplastic proliferation of the epithelium. The frequency of foci of such proliferation in the early stages of the experiment was close to that in cultures of rat embryonic lung, but in the later stages it reached the level of those changes in mouse embryonic lung (Table 1).

It is generally known that the appearance of tumors and, in particular, tumors of the lungs induced by chemical carcinogens in vivo is usually preceded by the development of diffuse and focal areas of hyperplastic proliferation [3, 5, 9]. During investigation of the early changes induced by chemical carcinogens in sensitive target tissues, in some cases various types of injury to the cells are discovered and these precede the development of precancerous foci of proliferation. As a rule, under these circumstances the sensitivity of the tissues to the repeated action of toxic concentrations of the carcinogenic substances is reduced; this is found, for example, during the induction of tumors of the liver, skin, and connective tissue [2, 13].

The results of these experiments show that under the conditions of organ culture of embryonic lung tissue, repeated exposure to NMU induces processes in that tissue that are evidence of the initial stages of tumor growth. The higher rate of survival of the experimental and of the intact cultures evidently also reflects changes in the properties of the explanted tissue in the course of tumor evolution. It is important to note that, despite definite differences in the reaction to NMU, these patterns are observed in the lungs of both rodents and man.

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