from the same donors are given in Table 3. It will be clear from Table 3 that EFCF for trypsinized bone marrow was significantly higher than for mechanically disaggregated bone marrow. The presence of the additional feeder increased EFCF, and for cells of trypsinized and nontrypsinized bone marrow it was ($\cdot 10^{-4}$), 11.3 ± 1.9 and 0.7 ± 0.1, respectively.

Table 4 gives the results of experiments in which the effects of trypsinization of bone marrow fragments or of cell suspensions obtained by mechanical disaggregation were compared. The bone marrow from the left femur was first trypsinized, then passed through syringes, whereas marrow from the right femur of the same donors was passed first through syringes and, later, half of the resulting suspension was trypsinized. According to the results of these experiments EFCF for cells obtained by trypsinization of bone marrow fragments was significantly higher and for cells isolated by mechanical disaggregation; subsequent treatment of the mechanically disaggregated bone marrow by trypsin, moreover, did not increase EFCF.

The increase in EFCF during trypsinization of mouse bone marrow was thus not connected with activation by trypsin of the colony-forming properties of the already disaggregated cells, but with additional release of FCFC, which either are not released by mechanical disaggregation of bone marrow, or are injured. The experiments showed that the presence of hematopoietic cells has a stimulating action on the development of mouse stromal colonies, and their colony-stimulating activity is still preserved after irradiation in a dose of 60 Gy, i.e., it evidently is independent of proliferation of feeder cells. By trypsinization and the use of an irradiated feeder it is possible to increase EFCF for mouse bone marrow substantially by comparison with EFCF values achieved previously [2].

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TRANSPLACENTAL EFFECT OF METHYLCOBALAMIN ON GROWTH OF MOUSE EMBRYONIC KIDNEY TISSUE IN ORGAN CULTURE

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The effect of methylcobalamin (MC) on growth of embryonic tissues is due to its functional role as coenzyme for methionine synthetase (EC 2.1.1.13), which controls the formation of active forms of folate required for the intensive turnover of C_1 compunds in proliferating cells in mammals and man [11]. The cellular level of cobalamin-dependent methionine synthetase in embryonic tissues is evidently an important parameter determining their rate of growth. During human embryogenesis the highest level of methionine synthetase is observed in the fetal tissues and the serum level of MC (the main transport form of the cobalamins) is high [9, 12]. The necessary supply of MC to the fetal tissues is ensured by means of placental receptors and different classes of transcobalamin in the blood serum. Intensive uptake of cobalamins by em-

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TABLE 1. Transplacental Action of MC of Growth of Organ Cultures of Embryonic Kidneys on DBA/2 Mice

Experimental conditions	Number of explants	Frequency of hyperplastic changes in renal epithelium, %				Frequency of destructive changes %		
		structural outgrowths	Diffusive hyperplasia of epithelium of convoluted tubules	Focal hyperplasia of epithelium	Cyst-like structures	Changes absent	With central necrosis	With replacement of central necrosis
Control	89	4,5	4,5	—	3,4	30,4	69,6	—
MC	103	16,5	55,3	7,1	13,6	27,2	42,7	30,1
		$P<0,05$	$P<0,001$	$P<0,01$	$P<0,01$		$P<0,001$	$P<0,01$

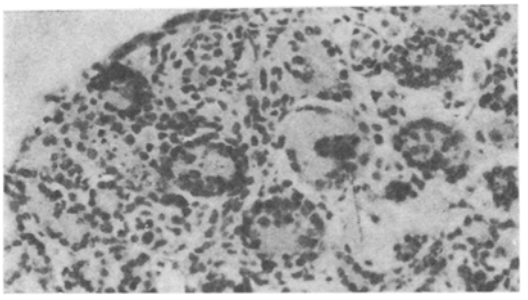

Fig. 1. Explant of embryonic kidney of intact mouse: tubular structures well preserved, against a background of slight necrotic changes (10th day of culture; 125 ×).

bryonic cells is determined by their increased requirement of methionine, essential for synthesis of polyamines and protein. Various aspects of the modifying action of MC on tumor growth have been investigated in recent years [6]. However, the transplacental influence of MC on growth of embryonic tissues and the possibility of its action in transplacental carcinogenesis have virtually not been studied.

This paper gives the results of a study of the transplacental action of MC on growth of embryonic tissues during organ culture. An organ culture of embryonic mouse kidney tissue, sensitive to the transplacental action of biologically active substances, including vitamins and hormones, and also of chemical carcinogens [8], was used as the experimental model.

EXPERIMENTAL METHOD

To create a high cobalamin concentration in the fetal tissues, MC was injected intramuscularly in a dose of 2.5 mg/kg at intervals of 48 h into female DBA/2 mice from the 14th through the 30th day of pregnancy. The total dose of MC was 10 mg/kg. The total serum cobalamin level was investigated in the mice on the 21st day of pregnancy by a microbiological method [2]. Embryonic kidney tissue from 21-day fetuses was cultured by the method in [1]. RPMI-1640 nutrient medium (50%), calf embryonic serum (20%), chick embryonic extract (25%), 40% glucose solution (3 ml to 100 ml of medium), 0.3% L-glutamine, 2% HEPES (2 ml/100 ml medium), and monomycin (100 U/ml) were used. At different times of culture (from the 4th to the 18th days) explants were studied histoautoradiographically in serial paraffin sections stained with hematoxylin and eosin. ^{3}H-Thymidine was added to the nutrient medium in a single dose of 1 μCi/ml (specific activity 1940 TBq/mole) 1 h before fixation of the explants. Organ cultures of fetal embryonic kidneys obtained from intact females were analyzed at the same time. Altogether 192 explants of mouse kidneys were investigated. To estimate the transplacental action of MC the frequency of hyperplastic changes in the renal epithelium and the number of proliferating cells (labeling index LI) were determined by analysis of at least 1000 epithelial cells in each explant. The frequency of degenerative and regenerative changes in the organ cultures was determined at the same time. The results were subjected to statistical analysis by Student's t test.

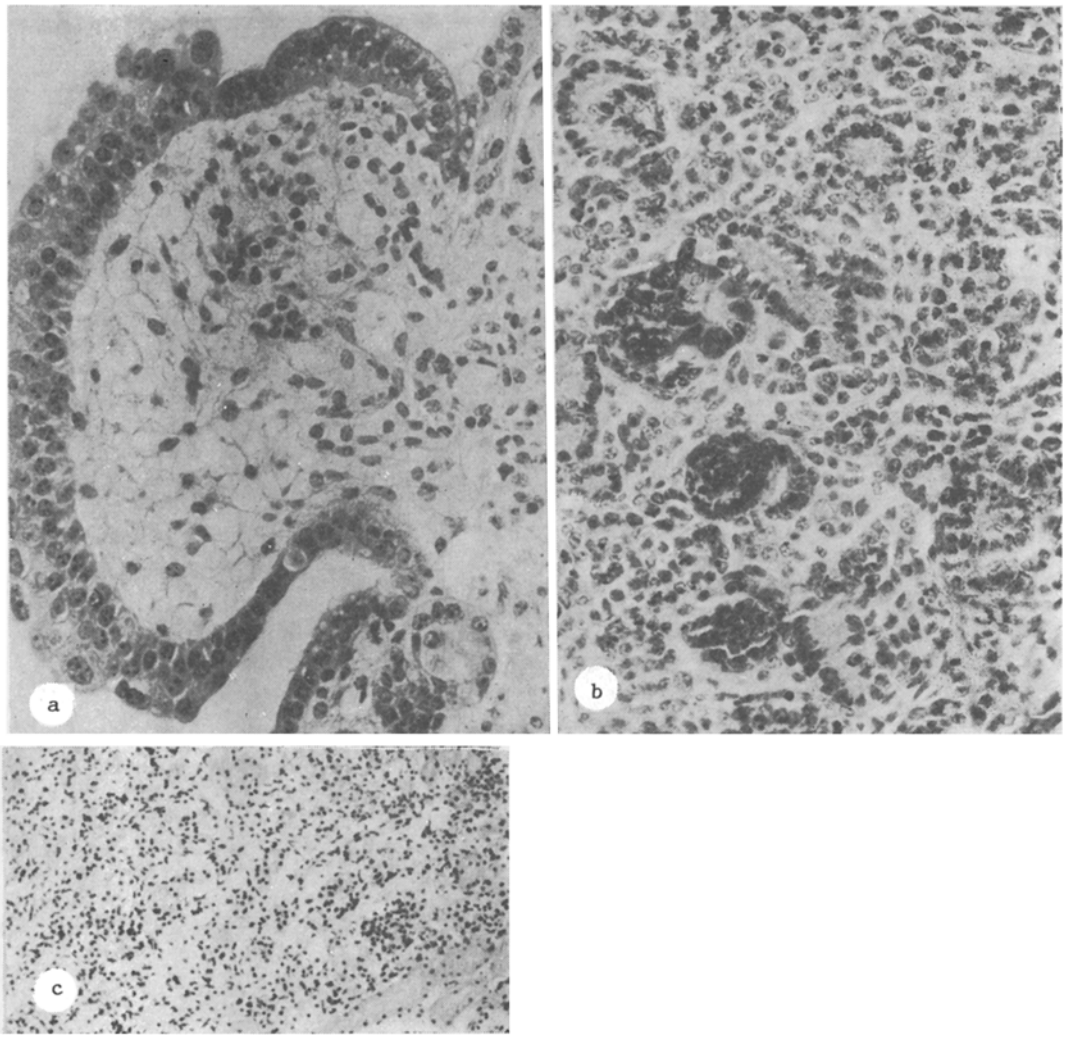

Fig. 2. Transplacental action of MC: a) general appearance of structural outgrowths covered with epithelial capsule (7th day of culture), 125 ×; b) diffuse hyperplasia of epithelium of convoluted tubules (10th day of culture), 300 ×; c) regenerative changes in zone replacing central necrosis (12th day of culture), 125 ×.

EXPERIMENTAL RESULTS

MC, administered in prenatal period, had a marked growth-stimulating action on organ cultures of the mouse embryonic kidney. This was reflected in the higher rate of survival of the explants, due to a marked decrease in the frequency of degenerative changes. Throughout the period of culture, for instance, the number of explants with marked zones of primary central necrosis was 42.7% compared with 69.6% ($P < 0.001$) in the control (Table 1). A significant feature distinguishing the transplacental action of MC on growth of mouse embryonic kidney tissue was the development of regenerative changes in the late stages of culture in zones replacing the central areas of necrosis. The frequency of these regenerative changes was 30.1% (Table 1). Regenerative changes in organ cultures of kidneys from intact mice did not appear at any time during culture (Fig. 1). Regeneration in zones replacing central necrotic areas took place as the result of active proliferation of epithelial and connective-tissue cells, and the number of DNA-synthesizing cells in these regions of the kidney explants was 7.3% (Fig. 2c).

During the transplacental action of MC, a higher rate of survival of the organ cultures was accompanied by an increase in the frequency of development of hyperplastic changes. Among the hyperplastic changes observed in the kidney organ cultures those most frequently found were structural outgrowths, or areas forming zones of growth at the periphery of the explants, and diffuse hyperplasia of the epithelium of the convoluted tubules (Fig. 2a, b). After prenatal administration of MC the number of structural outgrowths in the kidney explants was 3.9 times greater than in the control cultrues ($P < 0.05$). The number of epithelial cells synthesizing DNA in the zones of growth was $4.7 \pm 0.7\%$ compared with $3.1 \pm 0.6\%$ in the control. The fre-

quency of development of diffuse hyperplasia in the epithelium of the convoluted tubules under the influence of MC also was significantly increased to 55.3% compared with 4.5% in kidney explants from intact mice ($P < 0.001$). Against the background of marked diffuse hyperplasia, small foci of proliferation, evidently consisting of actively proliferating epithelial cells of the convoluted tubules, were found in 7.1% of explants. In addition, after administration of MC the number of cyst-like structures in the kidney explants was three times greater than in the control cultures ($P < 0.01$).

Mouse embryonic kidney tissue, under conditions of organ culture, is thus highly sensitive to the transplacental action of this cobalamin coenzyme. When MC was administreed prenatally, definite hyperplastic changes developed in the kidney explants, and were mainly observed at the periphery in the zones of growth, and were characterized by an increase in the number of structural outgrowths and by the development of diffuse hyperplasia in the epithelium of the convoluted tubules, and of regenerative changes in zones replacing the central areas of necrosis. Similar hyperplastic changes were observed in explants of intact mouse kidney, but the frequency of their development was much lower. After administration of MC, incidentally, the cobalamin concentration in the mouse blood serum was significantly raised on the 21st day of pregnancy, to 51.7 ng/ml compared with 0.7 ng/ml in intact females.

The ability of MC to stimulate proliferation of human embryonic fibroblasts *in vitro* as the result of an increase in the number of cells in the population entering the S-phase of the mitotic cycle was demonstrated by the writers previously [3, 4]. The stimulating effect of MC on growth of embryonic tissues was evidently due to a change in activity of methionine synthetase and the enzymes of polyamine biosynthesis: ornithine and SAM-decarboxylases. Linear correlation of the growth constant and the cellular methionine synthetase level was observed in a culture of normal rat liver epithelial cells during culture in methionine-deficient medium [13]. Administration of MC *in vivo* also stimulates growth of some transplantable mouse tumors: mammary gland adenocarcinoma 755, adenocarcinoma of the large intestine, RSHM-5 carcinoma of the cervix uteri [7]. The level of cell proliferation in target tissues is known to be one factor modifying the action of chemical carcinogens [10]. This evidently explains the increased sensitivity of embryonic tissues to transplacental carcinogenic action. The stimulating effect of MC on growth of mouse kidney tissue in the prenatal period, revealed by the present investigation, is an important mechanism modifying the transplacental action of chemical carcinogens.

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