The results of these experiments thus showed that chemical desympathization does not affect Mg-ATPase activity of erythrocyte membranes. However, when a myocardial infarct is produced after desympathization of the animals, this enzyme activity reveals certain special features which, on the basis of data in the literature, can be interpreted as an indication of a disturbance of erythrocyte membrane structure. This view is confirmed by the increase in LPO induced in erythrocyte membranes of desympathized animals, which is particularly marked in the acute period of myocardial infarction.

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INVESTIGATION OF PENETRATION OF TRITIATED CYCLIC AMP INTO VARIOUS MOUSE TISSUES

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The question of permeability of cèll membranes to tritiated cyclic AMP (cyclic-AMP-^{3}H) [4, 5, 7, 11, 12] has not yet been settled. Meanwhile cyclic AMP is known to have a role in the regulation of cell metabolism, and, in particular, during tumor growth [2, 9], and disturbances of extracullular cyclic AMP metabolism have been found in cancer patients [8]. Accordingly, in the investigation described below, penetration of cyclic AMP-^{3}H was studied into certain organs and tissues of intact mice and of mice with transplanted tumors (Ehrlich's carcinoma and sarcoma 180).

EXPERIMENTAL METHOD

Experiments were carried out on 60 male SHR mice weighing 22-24 g. Penetration of cyclic AMP-^{3}H into the tissue was studied simultaneously in intact animals and in mice with a trans-

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TABLE 1. Accumulation of Cyclic AMP-^{3}H in Organs and Tissues of Mice (M ± m)

Group of animals	Time after injection of cyclic AMP-^{3}H, min	Liver	Lungs	Adipose tissue	Muscle tissue	Intestine
Intact mice (n = 20)	30	107311±7388 / 100	14157±4570 / 100	11289±4951 / 100	2673±1172 / 100	660±215 / 100
	120	62826±4406 / 100	13645±479 / 100	16905±6952 / 100	5709±1667 / 100	924±33 / 100
Mice with Ehrlich's carcinoma (n=20)	30	32918±14495* / 30,8	1337±743* / 9,4	1716±198 / 15,2	1106±182 / 41,4	924±264 / 140,0
	120	11748±4521* / 18,5	1238±1171* / 9,1	1327±60* / 7,8	1255±528* / 22,0	1980±725 / 214,2
Mice with sarcoma 180 (n = 20)	30	1340±450* / 1,2	820±380* / 5,8	410±290* / 3,6	570±80 / 21,3	1660±60* / 251,5
		1560±120* / 2,5	540±250* / 4,0	360±80* / 2,1	340±150* / 6,0	2192±396* / 237,2

Legend. Data in numerator given in cpm/g wet weight of muscle tissue, in denominator as percentages (control — intact mice — taken as 100%). *) Difference significant compared with corresponding control ($P < 0.05$).

planted tumor on the 14th day after transplantation, which was carried out subcutaneously by the usual method. Cyclic AMP-^{3}H (specific activity 800-900 GBq or 20-30 Ci/mmole, USSR product) was injected intraperitoneally in a dose of 2 μ Ci (70×10^{-6} to 80×10^{-6} GBq) per mouse. The animals were decapitated 30 and 120 min after labeling, and the liver, lungs, intestine (a segment of the ascending colon measuring 2-3 cm), and muscle (femoral muscles) and adipose (epididymal) tissue were removed and placed for 15 min in 0.15 M NaCl at 4°C. Tissue from five animals of each group was pooled and kept at −20°C for 48-72 h. After thawing, the material was cut into pieces with scissors in the cold and 1 g of tissue was homogenized in a glass homogenizer, with pestle revolving at a speed of 3000 rpm for 60 sec, in 1 ml distilled water (0 ± 1°C). The homogenizer was rinsed with a further 1 ml of cold water and the pooled homogenate was transferred for 60 sec to a boiling waterbath, 0.05 ml of 0.08 M $CaCl_2$ was added, and the mixture was centrifuged at 2000 g for 15 min (18°C). The supernatant (cell-free protein-free extract) was kept at −20°C. Cyclic AMP-^{3}H was identified and the quality of the preparation verified before the beginning of the investigation by thin-layer chromatography on DEAE-cellulose [3] in a system of isopropyl alcohol and ammonia (1:2). The tissue homogenate (60 μl) and "cold" cyclic AMP (from Fluka, Switzerland) (10 μl, 30 μg) were applied to the start. Cyclic AMP-^{3}H was located with the aid of an ultrachemiscope, and the corresponding areas of DEAE-cellulose were removed with a scalpel, transferred to flasks containing dioxan scintillator, and the radioactivity of the samples was measured on a Mark II counter (from Nuclear Chicago, USA). Activity was expressed in cpm/g wet weight of tissue.

EXPERIMENTAL RESULTS

The averaged results of the two series of experiments to study accumulation of cyclic AMP-^{3}H in the mouse tissues are given in Table 1. They show that cyclic AMP-^{3}H accumulated in intact mice in the liver, lungs, adipose and muscle tissues, and intestine (in diminishing order). This same order (with some exceptions) was preserved in mice with Ehrlich's carcinoma, whereas in mice with sarcoma 180 the greatest accumulation of cyclic AMP was observed in the intestine. Accumulation of cyclic AMP-^{3}H in the liver, lungs, and adipose and muscle tissues of the mice with tumors also was reduced compared with the control, whereas an increase in accumulation of cyclic AMP was observed in the intestine of mice with sarcoma 180 and there was a tendency for cyclic AMP accumulation to increase in the intestine of mice with Ehrlich's carcinoma.

These results thus indicate that cyclic AMP penetrates into cells of various mouse tissues and that penetration of this cyclic nucleotide into some tissues of mice with tumors is reduced. Among the possible causes of these differences in the character of accumulation of cyclic AMP-^{3}H observed in intact mice and mice with transplantable tumors, it is possible to mention increased excretion of labeled cyclic AMP from mice with tumors [1], the systemic action of the neoplasm on the host organism [6], a reduction in permeability of the plasma membranes for cyclic AMP and the consequent retention of the latter in the blood stream, and also

increased destruction of cyclic AMP due to increased phosphodiesterase activity. However, determination of cyclic AMP-^{3}H in the blood revealed no significant didferences between intact animals and animals with tumors, in the same way as no increase of radioactivity was observed in the zone of the chromatograms from the latter corresponding to the localization of 5'-AMP. To explain the results, the cyclic AMP-binding capacity of cell membranes and of cytosol proteins and the accumulation of labeled cyclic AMP in tumor tissue must be investigated, the possibility of a relative redistribution of cyclic AMP leading to its selective accumulation in certain tissues (in particular, in the intestine, as was observed in the present experiments; moreover, it is not clear whether this accumulation is linked with the excretory function of the intestine, with its possible special role in cyclic AMP metabolism, or with other causes) must be studied, and, in addition, the role of certain factors connected with the state of the tumor-bearing organism and, in particular, disturbances of lipid metabolism, must also be investigated. Mice with Ehrlich's carcinoma are known to have marked hyperlipidemia [10], and this is one of the factors which contributes to the lowering of reactivity of the cyclic AMP system to functional stimulation [8]. In this respect it must also be pointed out that, according to the writers' preliminary observations, a reduction in accumulation of cyclic AMP-^{3}H was observed in certain tissues of mice with hyperlipidemia induced by Triton WR 1339, and in rabbits fed for a long time with cholesterol.

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