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POSSIBILITY OF USING GUINEA PIGS FOR STUDYING THE CARCINOGENIC ACTIVITY OF SOME ENDOGENOUS SUBSTANCES

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The tryptophan metabolites 3-indolylacrylic acid and 2-aminoacetophenone, whose carcinogenic activity has been demonstrated by experiments on inbred mice, were injected into noninbred guinea pigs. Both substances induced tumors in the animals of the experimental groups earlier than in the control and the tumors differed significantly in their morphology from those in animals of the control groups, evidence that both compounds have a carcinogenic effect. The results indicate that guinea pigs can be used to study the carcinogenic activity of weak carcinogens of the endogenous class.

KEY WORDS: guinea pigs; tryptophan metabolites; carcinogenic activity; endogenous carcinogens.

The extremely low frequency of spontaneous neoplasms in guinea pigs [6, 11] and the fact that their blood serum has an inhibitory effect on certain transplantable mouse tumors [8, 13] has led to the view that guinea pigs are resistant to induction of tumors and, as a result, the term "carcinoresistance" has become firmly attached in the specialized literature to the animals of this species [5, 10, 12].

Shabad [2], however, has shown that guinea pigs are not absolutely refractory to the carcinogenic activity of dibenzanthracene, and later work has revealed the high sensitivity of guinea pigs as a species to nitroso compounds, aflatoxins, cycasin, hormones, etc. [4, 5, 9, 12, 14, 15].

The object of this investigation was to use guinea pigs in an attempt to study the carcinogenic activity of tryptophan metabolites, which are found in an increased concentration in the urine of patients with leukemia and have a carcinogenic effect in experiments on inbred mice [1].

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred guinea pigs of both sexes obtained from the Kryukovo nursery at the age of 2 months and kept on a standard diet in individual cages. The animals were divided into

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TABLE 1. Location and Morphological Features of Spontaneous and Induced Tumors of Guinea Pigs

Location	Morphological features	Number of tumors		
		3IAA	2AAP	control
Blood system	Lymphatic leukemia	1	—	2
Adrenals	Adenoma of the cortex	—	—	3
	Carcinoma of the cortex	—	—	1
Pancreas	Islet-cell tumor	1	—	—
Ovaries	Dermoid cyst	—	—	1
Uterus	Leiomyoma	—	2	—
Subcutaneous cellular tissue	Angiosarcoma	—	1	—
Mesentery	Angiosarcoma	1	—	—

three groups. The two experimental groups of guinea pigs received 2-aminoacetophenone (2AAP; 20 animals) and 3-indolylacrylic acid (3IAA; 30 animals); the remaining 40 guinea pigs served as the control. Both compounds were injected subcutaneously in a dose of 30-50 mg in 0.5 ml distilled water at intervals of 2-3 days for 3-4 months. The total dose was 550 mg of 3IAA and 740 mg of 2AAP, which was based on the results of similar experiments on mice. All animals of the experimental and control groups were kept under observation until natural death. The animals were examined post mortem and material was taken from tumors of the liver, kidneys, spleen, lymph nodes, lungs, uterus, ovaries, and bladder and fixed in 10% formalin solution. Pieces of the organs and tissues were embedded in celloidin or paraffin wax and sections were stained with hematoxylin–eosin and also, where necessary, with picrofuchsin mixture, by Foot's method, with azan, and by other special staining methods. Hematological tests (red and white cell counts, differential counts) were carried out periodically on all the animals for a diagnosis of hemoblastoses during life.

EXPERIMENTAL RESULTS

Among animals of the control group 7 tumors were found in five guinea pigs (12.5%). The number of neoplasms in the two experimental groups was practically identical with that in the control group: in 6 of the 50 animals (12%). However, differences were found in the times of discovery of the neoplasms, and, in particular, in the age of the animals at which the first tumor was diagnosed. Whereas in the control group tumors appeared in all five guinea pigs mainly after the age of 4.5 years (the first tumor appeared at 4 years 3 months), in the group of animals receiving 3IAA the first tumor appeared at the age of 2.5 years, and in the group receiving 2AAP at 3.5 years. The mean age of the animals with tumors in the experimental groups was 3 years 11 months and in the control 4 years 7 months. Furthermore, the tumors in the animals of the experimental groups differed significantly from those in the guinea pigs of the control group (Table 1). Whereas in the control group only neoplasms belonging to the endocrine system appeared, namely a dermoid cyst of the ovary, carcinoma and three adenomas of the adrenal cortex (Fig. 1a), the tumors in the animals of the experimental groups were considerably varied. They included two leiomyomas of the uterus (one with proliferation and mitoses), an angiosarcoma of the mesentery and subcutaneous cellular tissue (Fig. 1b), and a malignant islet-cell tumor (Fig. 1c, d). An analysis of the literature shows that neoplasms such as malignant islet-cell tumor and angiosarcoma, diagnosed in the animals of the experimental groups, are extremely rare or have not been described at all among the spontaneous tumors of guinea pigs [6, 11]. On this basis it can be confidently concluded that these tumors arose as the result of induction by the tryptophan metabolites tested, by contrast with lymphatic leukemias, which were found among both the control and experimental animals.

The results of the experiments on noninbred guinea pigs thus reveal definite carcinogenic activity of 2AAP and 3IAA. No significant differences could be found between the carcinogenic action of the two substances. The guinea pigs were found to be less sensitive to endogenous tryptophan derivatives than inbred mice of strains C57BL and CC57BR in analogous experiments [1]. It can accordingly be postulated that the total doses of the substances tested were not optimal for the manifestation of their true carcinogenic effect in guinea pigs, although the use of the optimal dose is one of the most important conditions for the action of carcinogenic agents [3]. The possibility cannot be ruled out that guinea pigs also are less sensitive to the car-

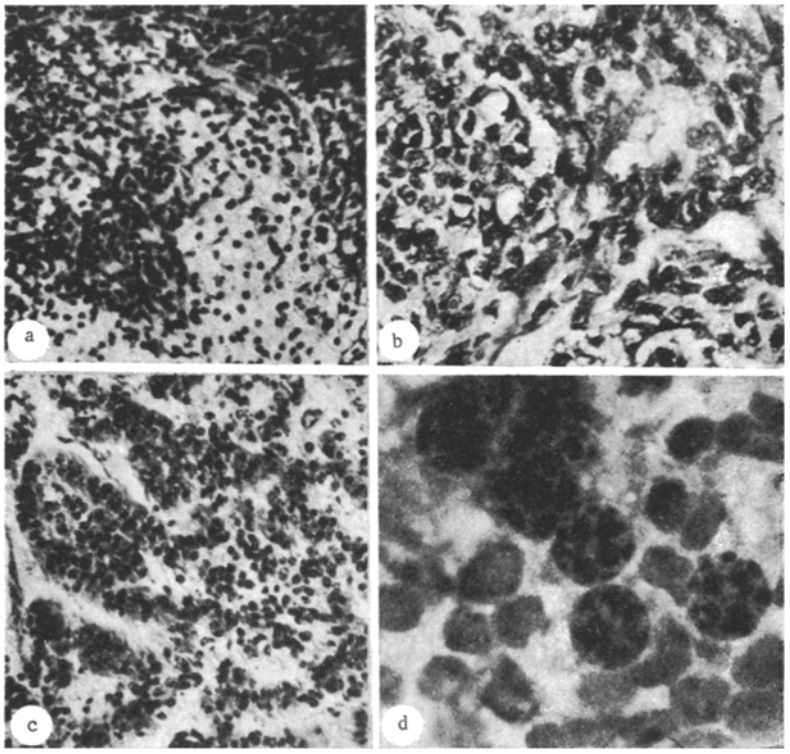

Fig. 1. Spontaneous tumors of guinea pigs and tumors induced by tryptophan metabolites: a) pale-cell adenoma of adrenal cortex in control female aged 4 years 3 months. Hematoxylin–eosin, 112.5×, b) angiosarcoma of subcutaneous cellular tissue in male aged 3 years 11 months receiving 740 mg 2AAP subcutaneously. Vascular spaces surrounded by pale polymorphic cells resembling endothelium can be seen. Hematoxylin–eosin, 200×, c) malignant islet-cell tumor in male aged 4 years 8 months receiving 550 mg 3IAA subcutaneously; tumor solid in places, in others trabecular in structure; sinusoids at foci of proliferation of delicate fibrous connective tissue between trabeculae. Hematoxylin–eosin 112.5×, d) cytological preparation of same tumor; large clumbs of chromatin giving the nuclei a distinctly spotted appearance characteristic of nuclei of cells of islets of Langerhans. Azure–eosin, 600×.

cinogenic action of tryptophan metabolites by analogy with the resistance of the animals of this species to some clinical carcinogens. Meanwhile their high sensitivity to nitroso compounds, to cycasin, and so on, also points to certain metabolic peculiarities in the animals of this species.

It has recently been shown that the resistance of guinea pigs to induction of tumors by 2-acetylaminofluorene is connected with the special features of its metabolism in these animals. Their carcinoresistance is also linked with a high blood serum L-asparaginase level, a characteristic feature of guinea pigs alone [8, 13]. Close correlation has been found between fluctuations in the level of activity of this enzyme and the inhibitory effect of guinea pigs' blood serum in experiments with transplantable tumors of mice and rats [8, 13].

The age of the guinea pigs plays an important role in the onset of spontaneous neoplasm. This fact was confirmed by the present experiments also, for they showed that practically no tumors were found in the control animals before the age of 4 years. In this connection it should be noted that the inhibitory effect of the blood serum of guinea pigs aged 4 years is much weaker than that of guinea pigs aged 1 year [7], further indirect evidence of the possible connection between the refractoriness of these animals to tumors and their high L-asparaginase level.

The results of these observations, combined with data in the literature, thus suggest the existence of state of relative resistance in guinea pigs to the induction of tumors by certain carcinogens. The discovery of the carcinogenic activity of some of the tryptophan metabolites which have been investigated indicates that animals of this species can be used to study the carcinogenic properties of endogenous substances.

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TRANSPORT OF Ca^{2+} IONS IN THE MITOCHONDRIA OF EHRLICH'S ASCITES TUMOR CELLS

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The pattern of Ca^{2+} accumulation by tumor mitochondria (MC) was investigated under various experimental conditions. In the absence of penetrating anions tumor MC were shown to take up Ca^{2+} in only one fifth the amount taken up by liver MC. In the presence of acetate this difference was greater still. Inorganic phosphate (P_{in}) abolished the observed defects of Ca^{2+} transport and increased the Ca^{2+} capacity of the tumor MC considerably. By contrast with liver MC, P_{in} also had a stabilizing effect on membrane permeability of the tumor MC; this may be the cause of the increasee Ca^{2+} capacity of these MC.

KEY WORDS: tumor mitochondria; Ca^{2+} transport; membrane permeability.

A disturbance of Ca^{2+} homeostasis in the cells is an essential stage in the development of pathological processes. It has been shown, in particular, that the mitochondria (MC) of tumor cells can take up and retain unusually large concentrations of Ca^{2+} [4]. However, the causes of the increased Ca-accumulating capacity of the MC of tumor cells have not been studied.

The object of this investigation was to study Ca^{2+} accumulation by tumor MC under different experimental conditions.

EXPERIMENTAL METHOD

An Ehrlich's ascites tumor was induced in sexually mature male albino mice by intraperitoneal injection of 10^6 cells of a diploid strain of Ehrlich's ascites carcinoma into each animal. The cells were harvested 10 days after inoculation and were separated from the ascites fluid by centrifugation. MC were isolated by a

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