LITERATURE CITED

1. P. A. Dyban, in: Tumor Growth as a Problem in Developmental Biology [in Russian], Moscow (1979), p. 174.
2. I. Damjanov and D. Solter, Curr. Top. Pathol., 95, 69 (1974).
3. K. Illmensee, in: Genetics, Mosaics, and Chimeras in Mammals, L. B. Russell, ed., New York (1978), p. 3.
4. F. Jacob, Immunol. Rev., 33, 3 (1977).
5. G. R. Martin, L. M. Willey, and I. Damjanov, Dev. Biol., 61, 230 (1977).
6. M. V. McBurney, J. Cell. Physiol., 89, 441 (1976).
7. H. Quastler and F. J. Sherman, Exp. Cell Res., 17, 217 (1959).
8. M. J. Rosenstraus, C. Sundell, and R. M. Liskay, Dev. Biol., 89, 516 (1982).
9. L. C. Stevens, Dev. Biol., 21, 364 (1970).

GLUCONEOGENESIS IN ANIMALS WITH EXPERIMENTAL TUMORS TREATED BY HYDRAZINE SULFATE

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KEY WORDS: hydrazine sulfate; gluconeogenesis; treatment of tumors; antitumor action.

Hydrazine sulfate (HS) has antitumor activity under experimental conditions [6, 11] and is an effective agent in clinical oncology [10]. It has no marked side effects, such as are characteristic of most known antitumor agents [3, 9]. It has been suggested [12] that HS goes some way toward normalizing carbohydrate metabolism, when disturbed during tumor growth, by sharply inhibiting gluconeogenesis. This effect is achieved through inhibition of phosphoenolpyruvate carboxykinase, the key enzyme of gluconeogenesis. As a result the vicious circle of glycolysis—gluconeogenesis—glycolysis, arising in malignant disease, which demands high energy expenditure and leads to the well-known cancer cachexia, is broken. The author cited gives indirect evidence in support of this hypothesis, but no direct experimental data on inhibition of gluconeogenesis during effective antitumor treatment with HS has hitherto been obtained.

The aim of this investigation was to study gluconeogenesis *in vivo* in the liver and kidneys (the main organs in which it takes place) of experimental animals with transplanted tumors after treatment with HS. The blood sugar also was determined to reflect changes in gluconeogenesis.

EXPERIMENTAL METHOD

Noninbred rats weighing 130-150 g with transplanted Zajdela's ascites hepatoma and noninbred albino mice with transplanted NK/LI ascites lymphoma were used. Daily intraperitoneal injections of HS (80 mg/kg) were given to the animals starting 24 h after transplantation; the control animals received 0.9% NaCl solution. This course of treatment leads after 7 ± 2 days to significant inhibition of tumor growth [5]. The rats were decapitated on the 7th-9th day after transplantation of the tumor, the mice on the 5th-7th day, and in all cases one day after the last injection of HS.

To assess gluconeogenesis *in vivo*, labeled glucose newly formed from [^{14}C]-2-alanine was determined. The labeled alanine was injected intraperitoneally (30 μCi into mice, 300 μCi into rats) and the animals were decapitated 1 h after injection of the isotope. Glucose was isolated by column ion-exchange chromatography [1]. Glucose was estimated quantitatively by the enzymic method with glucose oxidase [2]. Radioactivity in the samples was measured on a Mark 2 counter, using dioxin scintillator.

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TABLE 1. Formation of Labeled Glucose from [^{14}C]-2-Alanine after Treatment of Animals with a Course of HS (mean results of eight experiments)

Test object	Experimental conditions	Liver (cpm/g tissue ($\cdot 10^3$))	Kidneys (cpm/g tissue ($\cdot 10^3$))	Blood, cpm/ml ($\cdot 10^3$)
Rats with Zajdela's hepatoma	Control	14,9±23,6	52,4±14,3	79,1±13,5
	Treatment with HS	66,9±13,1	94,8±12,7	81,5±14,4
	P	<0,01	<0,05	=0,9
Mice with NK/LI lymphoma	Control	52,7±12,2	36,5±4,2	23,8±2,5
	Treatment with HS	21,5±3,5	51,2±2,2	39,4±3,2
	P	<0,02	<0,05	<0,01

TABLE 2. Glucose Content in Organs of Tumor-Bearing Animals after Course of Treatment with HS (mean results of six experiments)

Test object	Experimental conditions	Liver (mg/g tissue)	Kidneys (mg/g tissue)	Blood, mg%
Rats with Zajdela's hepatoma	Control	2,01±0,53	0,86±0,22	72,2±2,4
	Treatment with HS	1,83±0,2	0,56±0,16	62,5±0,5
	P	>0,1	>0,2	>0,1
Mice with NK/LI lymphoma	Control	2,37±0,74	0,87±0,09	70,1±13,2
	Treatment with HS	1,6±0,47	0,76±0,12	62,8±12,3
	P	>0,3	>0,4	>0,6

EXPERIMENTAL RESULTS

The results of the study of the intensity of gluconeogenesis in the liver and kidneys and data on the content of newly formed glucose in the blood after treatment with HS, reflecting this intensity, are given in Table 1.

It will be clear from Table 1 that the level of newly formed glucose in the liver of both rats and mice was reduced about by half. Hence it follows that effective treatment of the two chosen primary tumors with HS is accompanied by a reduction in the intensity of gluconeogenesis by half. In the kidneys, on the other hand, the level of newly formed glucose was approximately doubled, evidence of corresponding intensification of gluconeogenesis. The concentration of labeled glucose in the blood of rats with Zajdela's hepatoma remained unchanged, and this can be interpreted as overall balance of gluconeogenesis. The concentration of newly formed glucose in the blood of mice with NK/LI lymphoma was significantly increased. Among laboratory animals, it is mice which have the fastest rate of gluconeogenesis [4]. With this in mind it can be postulated that the increase in gluconeogenesis in the renal cortex of mice with NK/LI lymphoma not only compensates for the decrease in glucose formation in the liver, but actually causes an increase in the blood level of labeled glucose.

The results of the experiments on rats and, even more, those on mice do not confirm the original hypothesis on the mechanism of action of HS in the treatment of tumors. For a more detailed study of the therapeutic action of HS on the sugar concentration in the blood and organs of tumor-bearing animals, the results of the experiment described in Table 2 may be considered.

These results showed that treatment with HS caused no significant changes in the glucose concentration in the liver, kidneys, and blood of animals with experimental tumors. Irrespective of changes in the level of newly formed glucose in the liver and kidneys of the animals, and also in the blood of the mice, these do not correlate at all with the overall glucose concentration.

Considering the absence of therapeutic effect of HS under experimental conditions on phosphoenolpyruvate carboxykinase activity demonstrated previously [5] under similar experimental conditions, the data described in this paper are evidence against Gold's hypothesis [12], at least so far as the two tumors which we studied are concerned. In our opinion the mechanism of the therapeutic action of HS on malignant tumors must be sought in its effect on other biochemical stages. The resultant effect in this case will be determined by its action on several such stages, one of which, but by no means the most important, may be gluconeogenesis, since its inhibition would be revealed in any event in intact rats during the first few hours after administration of a toxic dose of HS, several times greater than the therapeutic dose which we used [13]. In particular, the action of HS on vitamin B_6 metabolism [8] and its effectiveness as an inhibitor of biotransformation [7] have been established by the present writers. This last effect causes changes in the level of endogenous metabolites, which could affect both the status of the tumor-bearing animal and also growth of tumor cells. The antimonoamine-oxidase effect of HS has also been demonstrated [8]. Considering the clinical picture during treatment with HS [10], we are inclined to regard this particular effect as the most important in the action of HS on tumor-bearing animals.

LITERATURE CITED

1. V. A. Blinova and V. S. Shapot, Vopr. Onkol., No. 3, 60 (1974).
2. V. K. Gorodetskii, in: Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), pp. 311-316.
3. L. A. Danova, M. L. Gershanovich, V. A. Filov, et al., in: Problems in Radiobiology and the Biological Action of Cytostatic Preparations [in Russian], Vol. 8, Tomsk (1977), pp. 192-193.
4. I. N. Kendysh, Usp. Sovrem. Biol., 86, No. 2 (5), 192 (1978).
5. V. S. Misheveva, T. M. Burova, and T. A. Goryukhina, Vopr. Onkol., No. 7, 71 (1980).
6. I. F. Seits, M. L. Gershanovich, V. A. Filov, et al., Vopr. Onkol., No. 1, 45 (1975).
7. A. V. Tret'yakov and V. A. Filov, Vopr. Onkol., No. 5, 94 (1977).
8. A. V. Tret'yakov, V. A. Filov, E. M. Ryazanov, et al., Vopr. Onkol., No. 6, 100 (1979).
9. V. A. Filov, A. N. Stukov, M. A. Blank, et al., Farmakol. Toksikol., No. 2, 203 (1978).
10. M. L. Gershanovich, L. A. Danova, V. A. Ivin, et al., Nutr. Cancer, 3, 7 (1981).
11. J. Gold, Oncology, 27, 69 (1973).
12. J. Gold, Nutr. Cancer, 1, 4 (1979).
13. P. D. Ray, R. L. Hanson, and H. A. Lardy, J. Biol. Chem., 245, 690 (1970).

DISTRIBUTION OF SURFACE FIBRONECTIN IN LOW- AND HIGH-DENSITY CULTURES OF NORMAL AND TRANSFORMED MOUSE FIBROBLASTS

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Fibronectin is a high-molecular-weight adhesive glycoprotein synthesized by cells of different tissues. It is present on the cell surface and in biological fluids [2, 5, 6]. Surface fibronectin is often deposited in the form of fibrillary polymers, and together with collagen and glycosaminoglycans it forms the external supporting matrix of the cells. It has been shown by lactoperoxidase iodination that malignant transformation (especially virus-induced) of connective-tissue cells is usually accompanied by partial or complete loss of

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