With the exception of BSA injections, all the procedures used delayed growth of mammary gland tumors at different times after their appearance. The most marked slowing of tumor growth was observed in the adrenalectomized rats, especially in those subsequently receiving thymosin. The rate of tumor growth in animals receiving BSA after the end of the DMBA injections was indistinguishable from that in the control.

The results of these experiments thus confirmed the view that the antitumor resistance of animals is enhanced by administration of thymosin in the presence of hypocorticalism. In the writers' view the effect of these therapeutic tactics on the development of induced mammary gland tumors in rats can be attributed to strengthening predominantly of cell-mediated immunity on account of removal of the limiting influence of glucocorticoids on the lymphoid system. Hypocorticalism in rats after adrenalectomy, it will be noted, is temporary in character for these animals have accessory adrenal tissue.

Considering that progressive growth of tumors is frequently accompanied by increased secretion of glucocorticoids [2, 7], the writers suggest that immunotherapy with thymosin against a background of temporary depression of hormone formation in the adrenal cortex (by means of chloditan, for example) will result in the more effective treatment of cancer patients.

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INSULIN AND GLUCOCORTICOID LEVELS IN ANIMALS WITH TRANSPLANTED TUMORS

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UDC 616-006-092.9-07:/616.154.379+616.154.453/-074

In mice with Ehrlich's ascites carcinoma and in rabbits with Brown–Pearce carcinoma, hypoglycemia was shown not to be the result of hyperinsulinemia. In rats with Zajdela's ascites hepatoma normoglycemia was the rule, and their blood insulin level did not change during growth of the tumor. The fall in the blood glucose level of rabbits with tumors was not due to weakening of the glucocorticoid function of the adrenals.

KEY WORDS: tumor; hypoglycemia; gluconeogenesis; insulin; glucocorticoids.

Profound hypoglycemia sometimes develops in cancer patients [8] and in animals with tumors [11, 14]. However, the pathogenetic mechanism of this phenomenon has not yet been explained. The view has been expressed that hypoglycemia in hormone-independent tumors is the result of excessive secretion of insulin of pancreatic or ectopic origin [7, 9]. Meanwhile there is conflicting evidence on the combination of hypogly-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 195-197, August, 1979. Original article submitted July 14, 1978.

0007-4888/79/8808-0894$07.50

TABLE 1. IRI Concentration (in micro-units/ml) in Plasma of Healthy Animals and of Animals with Tumors (M ± m)

Animals	Control	Experiment	
		9th-10th day	20th day
Rabbits	15,0±3,2	22,7±5,5	16,0±3,1
Mice	16,0±1,2	11,0±1,5*	—

*P < 0.02.

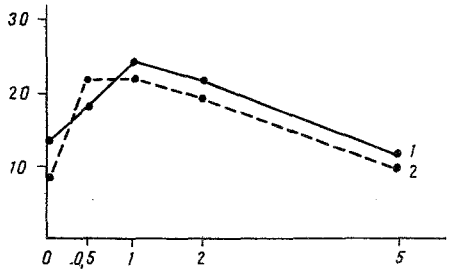

Fig. 1. Insulin curves in healthy mice (1) and mice with Ehrlich's carcinoma (2). 0) Time of injection of glucose. Abscissa, time of observation (in h); ordinate, IRI concentration (in micro-units/ml).

cemia with normo- or hypoinsulinemia [10, 12]. The resolving of this contradiction is very important for the understanding of the nature of metabolic changes developing in animals or patients with neoplasms.

The insulin and glucocorticoid levels in animals with transplanted tumors were studied.

EXPERIMENTAL METHODS

Experiments were carried out on mice, rats, and rabbits weighing 20-25, 150-200, and 2500-3000 g respectively. A suspension of Ehrlich's carcinoma cells was injected intraperitoneally, or of Crocker's sarcoma subcutaneously into albino mice, and tumor Ca-755 was injected subcutaneously into (CBA × C57BL)F_1 mice by the usual method. Zajdela's hepatoma was transplanted intraperitoneally into rats and Brown–Pearce carcinoma was transplanted into the testis of rabbits.

The blood glucose level of the animals was determined periodically by an enzymic method [4]. The serum concentration of immunoreactive insulin [13] and of 11-hydroxycorticosteroids (11-HCS), both protein-bound and free, biologically active forms [5], and the transcortin binding power (TBP) in vitro [3] also were investigated in mice with Ehrlich's carcinoma and in the rats and rabbits with tumors.

EXPERIMENTAL RESULTS

The writers found previously that in rabbits and mice, after transplantation of Brown–Pearce or Ehrlich's carcinoma respectively, there was a sharp decrease (by 40%) in the initial blood glucose concentration [1, 2]. Meanwhile Crocker's sarcoma and tumor Ca-755 in mice and Zajdela's hepatoma in rats grow against a background of normoglycemia [15]. In some of these animals with tumors, differing in their blood glucose levels, the concentration of immunoreactive insulin (IRI) was determined (Table 1).

As Table 1 shows, after transplantation of a Brown–Pearce carcinoma the IRI level in the rabbits' blood serum was unchanged. In mice with Ehrlich's carcinoma, hypoinsulinemia actually was observed although, admittedly, it was functional in character. In fact, after a single subcutaneous injection of 40% glucose solution in a dose of 10 mg/g into healthy and tumor-bearing mice the kinetics of their blood insulin concentration was practically identical (Fig. 1).

Deep hypoglycemia in rabbits and mice with tumors thus clearly arises not because of excessive secretion of insulin by the pancreas. Meanwhile the hypoinsulinemia in mice with Ehrlich's carcinoma is the

TABLE 2. 11-HCS Concentration and TBP in Plasma of Healthy Rabbits and of Rabbits with Tumors (M ± m)

Animals	Corticosteroids, μg%			TBP
	total	protein-bound	biologically active	
Healthy	5,5±0,27	4,9±0,19	0,7±0,01	16,2±1,09
With tumors				
10 days	7,9±0,66	6,1±0,60	1,9±0,46	17,4±1,09
P	<0,02	>0,1	<0,05	>0,5
20 days	5,8±1,04	4,8±0,87	1,0±0,18	22,0±0,77
P	>0,5	>0,5	>0,5	<0,02
30 days	8,2±1,0	6,3±0,65	1,9±0,26	24,1±1,58
P	<0,05	<0,05	<0,001	<0,01

Legend. Each group consisted of six animals.

result of functional insufficiency of the endocrine apparatus of the pancreas, for it is reactive in character and evidently develops secondarily in response to the hyperglycemia caused by the growing tumor.

The serum IRI concentration also was unchanged in animals in which normoglycemia was maintained all the time after transplantation of the neoplasm. Rats with Zajdela's hepatoma in fact died in the course of 5-6 days and their blood glucose fluctuated within narrow limits throughout the period of observation – from 61.0 ± 6.1 to 68.8 ± 4.2 mg% compared with a normal level of 67.1 ± 3.7 mg%. The serum insulin level of the healthy rats, however, was 20.9 ± 2.3 microunits/ml, rising to 24.6 ± 1.8 and 23.1 ± 3.9 microunits/ml by the 3rd and 5th days after transplantation of the tumor respectively ($P > 0.5$).

It can be suggested further that hypoglycemia in tumor-bearing animals is the result of hypofunction of the adrenal cortex or of corticosteroid insufficiency of "extra-adrenal" origin (increased binding with plasma transcortin). To test this hypothesis, the concentrations of 11-HCS and their fractions and the in vitro TBP were determined in the blood of healthy rabbits and of rabbits with Brown–Pearce carcinoma (Table 2).

It will be clear from Table 2 that the total plasma 11-HCS concentration in the rabbits with Brown–Pearce carcinoma was increased on the 10th and 30th days after transplantation of the tumor. By the 30th day of the experiment there was a tendency for the protein-bound plasma corticosteroid level to increase. These results correlate directly with the increase in the in vitro TBP. At the same time the biologically active corticosterone level, which is directly responsible for the regulation of metabolic processes, was increased in the tumor-bearing animals, despite the increase in protein capacity. In other words, complete realization of the increased TBP with respect to hormone binding did not take place in the animal, and this led to an increase in the levels of active forms of the hormones.

In the rabbits with Brown–Pearce carcinoma increased secretion of both total and biologically active glucocorticoids was thus observed, and not their hypoproduction. In other words, the hypoglycemia in rabbits with tumors is not connected with depression of the glucocorticoid function of the adrenals and an increase in TBP.

Consequently, the fall in the blood glucose level in the animals with neoplasms studied in these experiments was not the result of insulin hypersecretion, exhaustion of adrenocortical function, or a change in TBP. The hypoglycemia in the animals with tumors, as the writers showed previously [6], was the result of the action of the tumor as a "glucose trap," and also of inhibition of glucose synthesis from noncarbohydrate compounds. In fact, in mice with Ehrlich's carcinoma, the fall in the blood glucose level was found to correlate directly with the slowing of gluconeogenesis in the liver and kidneys. Similar correlation was found for the renal cortex in rabbits with Brown–Pearce carcinoma. Conversely, sharp stimulation of gluconeogenesis maintained the normoglycemia in mice with Crocker's sarcoma and tumor Ca-755, and also in rats with Zajdela's hepatoma [15].

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EFFECT OF DL-TRYPTOPHAN ON INDUCTION OF TUMORS BY DIETHYLSTILBESTROL IN *Rana esculenta*

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UDC 615.277.4:547.757

Diethylstilbestrol, injected subcutaneously into frogs (Rana esculenta), induced leukemias and tumors of the liver in 21.4% of animals. After combined administration of diethylstilbestrol and DL-tryptophan, tumors of these sorts developed in 52.6% of frogs. The differences in the frequency of leukemias in the animals of these groups were significant ($P < 0.05$). Administration of tryptophan alone did not induce tumors.

KEY WORDS: frogs; diethylstilbestrol; tryptophan; hemocytoblastosis.

Information on the potentiating effect of tryptophan with respect to the action of certain carcinogens [2, 9, 10, 13] and on the ability of its metabolites to induce leukemia [1, 3, 11] has been published. One of us (V. V. Khudolei) showed previously [6] that diethylstilbestrol, if given parenterally, induces hemocytoblastosis and hepatocellular carcinoma in the frog Rana temporaria.

The object of this investigation was to study the combined action of diethylstilbestrol and DL-tryptophan on Rana esculenta.

EXPERIMENTAL METHODS

The experiments were carried out on pond frogs (Rana esculenta), which are tailless amphibians, of both sexes aged 1-1.5 years. The 25 animals of group 1 received a subcutaneous injection of 100 μg diethylstilbestrol once a week in the dorsal region. The 40 frogs of group 2 received 75 mg of DL-tryptophan in 0.2 ml water by gastric tube once a day (5 times a week). The 40 frogs of group 3 received diethylstilbestrol and DL-tryptophan in the same way. The longest period of observation on the animals was 58, 122, and 65 days respectively. The liver, kidneys, and spleen from animals dying at different periods were subjected to morphological examination. Material was fixed in Bouin's fluid and paraffin sections were stained with hematoxylin-eosin. The numerical data were subjected to statistical analysis by Fisher's exact method and by the Wilcoxon–Mann–Whitney U criterion.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

Tumors of the hematopoietic system and liver were found in some of the animals in groups 1 and 3. When the hematopoietic organs were affected, the greatest macroscopic changes were observed in the spleen. The

Laboratory of Chemical Carcinogenic Agents, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 197-199, August, 1979. Original article submitted July 17, 1978.