action of the promoters in a culture of Jungarian hamster fibroblasts is exhibited irrespective of the organotropism of their action in vivo. Second, the promoters inhibited LY exchange independently of the character of their promoting action in vivo. In fact, TPA, anthralin, DDT, and BHT are powerful complete promoters [4, 7, 8, 11], whereas A23187 and mezerein are weak promoters of stages I and II, respectively [4, 8] and exhibit their activity only when their action is combined with that of other promoters.

The experiments thus showed that, in principle, tumor promoters with different types of action and with different degrees of organotropism can be detected by a method based on estimation of intercellular exchange of Lucifer yellow. The method has definite advantages that are essential for screening methods: the experimental results are highly reproducible (Table 2), only a few cells are needed for the test, and the results can be obtained within 1 to 2 days. The further study of the sensitivity and specificity of this method of detection of tumor promoters is important.

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CHANGES IN TISSUE GLYCOGEN RESERVES OF TUMOR-BEARING RATS AS A SIGN OF THE HYPOGLYCEMIC STRESS SYNDROME

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No systematic study has yet been made of changes in tissue glycogen levels in tumor-bearing animals. Data in the literature are difficult to compare and often reflect only the terminal periods of tumor growth.

This paper describes an attempt to study the glycogen content in the brain, skeletal muscles, and liver of animals during growth of malignant tumors. To analyze the particular

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TABLE 1. Changes in Glycogen Reserves in Tissues of Tumor-Bearing Animals during Growth of Neoplasm (M ± m)

Parameter	Fed healthy rats	Recipients of ZAH, time of observation, days				
		1	2	3	4	5
Serum glucose, mM	10,04±0,62 (12)	10,38±0,43 (10)	10,64±0,79 (10)	11,76±0,93 (10)	9,61±3,97 (10)	7,89±2,72 (13)
Glycogen, mg/g tissue						
Liver	57,92±5,86 (14)	45,70±7,87 (18)	51,77±7,58 (20)	50,32±8,68 (20)	14.61±10,30 (21)	6,34±3,90 (17)
Skeletal muscles	5,41±0,79 (13)	6,30±0,95 (20)	5,69±0,63 (19)	6,11±0,72 (20)	6,66±0,90 (20)	5,66±0,83 (19)
Brain	0,523±0,056 (8)	0,482±0,063 (7)	0,523±0,055 (8)	0,579±0,122 (8)	0,598±0,061 (8)	0,725±0,143 (8)

Parameter	Recipients of H-27, time of observation, days					
	5	10	15	20	25	30
Serum glucose, mM	9,60±0,21 (14)	10,12±0,23 (14)	10,24±0,42 (12)	10,14±0,28 (12)	9,30±0,27 (15)	A. 8,83±0,50 (6)
						Б. 11,74±0,22 (5)
Glycogen, mg/g tissue						
Liver	56,59±12,84 (29)	46,32±11,65 (20)	61,82±15,88 (16)	58,81±10,64 (16)	44,17±21,76 (16)	A. 18,29±12,62 (7)
						Б. 54,85±10,90 (6)
Skeletal muscles	5,30±0,66 (18)	5,49±0,67 (24)	6,14±0,79 (12)	6,34±0,74 (12)	7.47±1,05 (12)	7,99±1,01 (9)
Brain	0,541±0,072 (29)	0,519±0,051 (20)	0,583±0,083 (12)	0,552±0,050 (12)	0,658±0,098 (12)	0,734±0,155 (9)

Legend. A) Cachectic animal, B) animal with no signs of cachexia. Here and in Table 2, number of animals shown in parentheses.

features which could be responsible for anorexia, changes in the glycogen reserves were investigated not only in tumor bearing animals, but also in the corresponding tissues of healthy starving rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 80-140 g. A fast-growing Zajdela's ascites hepatoma (ZAH) was transplanted intraperitoneally, and a solid hepatoma 27 (H-27) was transplanted subcutaneously in the dorsal region.

Blood for analysis was taken from the abdominal aorta under ether anesthesia. The serum glucose level was determined with the aid of neocuproine [1].

The glycogen content in the skeletal muscles (hind limb) and liver was determined after desmolysis in 30% KOH, using the anthrone method [4]. Glycogen was extracted from the brain by the method in [3]. It was then hydrolyzed with γ-amylase, after which glucose in the sample was estimated by the glucose oxidase method [2]. Rabbit liver glycogen (from Serva, West Germany) was used as the standard.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The liver glycogen reserves of animals with ZAH were significantly reduced on the 1st day, but restored to normal on the 2nd-3rd days after transplantation of the tumor. However, after 4-5 days the glycogen content fell sharply, and in some animals it almost completely disappeared (Table 1).

The liver glycogen level in rats with H-27 fell until the 10th day after transplantation of the tumor. Later its content in the liver increased, and after 15 days it actually exceeded the control value. On the 30th day of growth of H-27, the animals could be divided into groups. Rats of group B, despite the large size of the tumor, were characterized by

TABLE 2. Changes in Glycogen Content in Brain, Liver, and Muscles of Rats during Starvation (M ± m)

Parameter	Fed healthy rats	Duration of starvation, days				
		1	2	3	4	5
Serum glucose, mM	10,04±0,62 (12)	6,62±0,67 (5)	7,73±3,30 (11)	9,86±2,67 (11)	9,15±1,95 (12)	8,43±1,24 (7)
Glycogen, mg/g tissue						
Liver	57,92±5,86 (14)	0,44±0,187 (11)	2,81±2,52 (9)	4,23±1,44 (12)	7,84±3,03 (11)	10,16±2,82 (14)
Skeletal muscles	5,41±0,79 (13)	4,24±0,73 (7)	4,80±0,69 (7)	4,59±0,63 (7)	4,18±0,49 (8)	4,40±0,57 (8)
Brain	0,523±0,056 (8)	0,421±0,031 (7)	0,564±0,069 (7)	0,532±0,054 (7)	0,515±0,084 (8)	0,450±0,056 (8)

normoglycemia and by a high glycogen concentration in the liver, whereas animals of group A exhibited hypoglycemia and lowered glycogen reserves. It is worth noting that the rats of group B, unlike those of group A, as a rule had no signs of cachexa.

The view is widespread in the literature that growth of a malignant tumor leads to a steady fall of the liver glycogen level in animals. However, the present results provide a fresh insight into the mechanism of mobilization of the liver glycogen in tumor-bearing animals. At the earliest times (the 1st day of growth of ZAH and the 5th-10th days of growth of H-27) the animal develops hypoglycemic stress, the intensity of which increases with growth of the neoplasm. This is shown by the fall of the liver glycogen reserves of tumor-bearing animals (Table 1). Under these circumstances normoglycemia was maintained, in our opinion, mainly due to increased glycogenolysis, for gluconeogenesis in rats with ZAH and H-27 has not yet been adequately stimulated at this time [5].

The results relating to the glycogen concentration in the rat liver during growth of ZAH and H-27 completely reflect the intensity of gluconeogenesis taking place in it. The maximal rate of gluconeogenesis from glycerol during perfusion of the liver of rats with ZAH was observed on the 2nd day, and in animals with H-27, on the 20th day after transplantation of the tumor [5]. Further growth of ZAH was accompanied by a sharp fall in the intensity of gluconeogenesis, or even its complete cessation, by the 5th day. A similar picture also was observed in rats with H-27 over a period of 25-30 days [5].

Data on the glycogen reserves in healthy starving rats were rather unexpected (Table 2). For instance, 24 h after deprivation of food, these animals showed complete exhaustion of their liver glycogen reserves. Later, however, a steady increase in them was noted, despite the absence of hyperglycemia. The phenomenon just described is in good agreement with the gluconeogenesis activated in these animals, and generating liver glycogen [5, 7]. Other workers also have reported elevation of the liver glycogen level in starving rats [10].

As regards the muscle glycogen level of the tumor-bearing animals, this remained within normal limits on the 5th-10th day in rats with H-27, and subsequently it rose until the 30th day after tumor transplantation. In animals with ZAH the muscle glycogen level remained within the limits characteristic of healthy fed animals, with a slight tendency to rise on the 1st-4th days after tumor transplantation. It is noteworthy that in animals with H-27 the muscle glycogen reserves on the 25th-30th days were higher than in recipients of ZAH. This difference was probably largely due to differences in the hormonal status of the animals of these groups [5, 6]. Similar stability of the muscle glycogen content was observed by other workers in animals with tumors [9], and it evidently reflects the special role of glycogen in the nutrition of this tissue. Reduction of the muscular activity of animals with tumors [8] (hypodynamia) may perhaps favor maintenance of the muscle glycogen reserves.

The brain is the main consumer of glucose in the body, but its glycogen reserves are low. No information could be found in the literature on the glycogen concentration in the brain of animals with tumors. As might be expected, hypoglycemia in healthy starving rats was accompanied by a fall in the brain glycogen reserves available for mobilization (Table 2).

Comparison of the brain glycogen concentration with the glucose level in the blood of rats with ZAH and H-27 revealed an unusual picture. It was found that in tumor-bearing animals hypoglycemia on the 5th day (ZAH) and on the 30th day (H-27), i.e., in the terminal

stage, was combined not with a fall, but on the contrary, with a rise of the brain glycogen level. It is difficult at the moment to explain this phenomenon.

Investigation of the glycogen reserves in tumor-bearing animals and comparison of the results with data for starving animals demonstrated the invalidity of the view that anorexia plays a role in the formation of the hypoglycemic stress syndrome. This syndrome is a component of metabolic stress, induced by a malignant tumor, which we regard as a multifactorial stress-inducing agent.

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