ON THE MULTIFORM RELATIONSHIPS BETWEEN THE TUMOR AND THE HOST

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I. Introduction

It is well known that cancer patients often die exhibiting dysfunction of vitally important organs, and disorders of regulation of metabolism and endocrine functions. In these cases death is caused directly by infections, disturbances in the balance between the coagulative and anticoagulative systems of the blood (thrombosis, thrombophlebitis, hemorrhage), and not from metastases that hinder the vital activities of the host.

All the above phenomena are often called complications that allegedly coincide with but have no direct relation to the main disease, that is, the progression of the malignant tumor, whereas actually they are a reflection and result of the pernicious action of the neoplasm on the homeostasis of the host.

The highest percentage of mortality is due to various infections. For example, according to the findings of Klastersky *et al.* (1972), who analyzed the results of the autopsies of 157 patients who succumbed to malignant neoplasms, in about 32% death was due directly to infection caused by gram-negative microorganisms resistant to accepted antibiotics. These authors note that the infection resulting in lethality was most

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commonly encountered not only in patients with acute leukemia, but also in patients with cancer of the upper respiratory passages, the gastrointestinal tract, and the urogenital organs without any signs of leukopenia.

According to the autopsies made over a period of four years (1971–1974) in the Cancer Research Center of the U.S.S.R. Academy of Medical Sciences, as many patients died of infectious complications (peritonitis, mediastinitis, pulmonary abscess, pericarditis, pneumonia, etc.) as of the progression of the main disease, staphylococci being the causative agents in 75% of the cases (Smolyanskaya and Grinenko, 1976).

According to the author of the "Annals of Internal Medicine" editorial (1973), "infection and cancer are old friends." From the data cited in the article it follows that the most common causes of death of oncologic patients are pneumonia and sepsis usually induced by nonvirulent white staphylococci and fungi. The mortality caused by different kinds of infection in oncologic patients sometimes reaches 65% (Sickless *et al.*, 1973).

The elevated sensitivity of oncologic patients to infection indicates a sharply marked immunodepression engendered by development of the tumor. Immunodepression in oncologic patients and animals with tumors was also repeatedly demonstrated by direct determination of the state of their cellular and humoral immunity (see, for example, Harris and Copeland, 1974). The possible causes of this surprising phenomenon deserve special attention and will be considered below (Section III,D).

Numerous cases are also encountered in which cardiovascular complications in patients with internal cancer are the direct causes of their death. The development of malignant tumors is often accompanied by peripheral neuropathies, myelopathies, polymyositides, and myasthenic syndromes, syndromes which sometimes precede the diagnosis of the main disease (Tyler, 1974). Twenty percent of all patients with polymyositis had malignant tumors (Pearson, 1966).

In a group of dermatomyositis patients past 40 years of age, cancer was diagnosed in 50% of the cases (Niebauer, 1974). Acanthosis nigricans, a rare dermatosis, in persons past 40 years of age denotes internal cancer in 60%–100% of the cases (Barriere, 1975; Storck, 1976), acanthosis nigricans being diagnosed in most of the patients *before* the appearance of tumor symptoms (Dedkova and Raben, 1977). More than 150 cases of severe hypoglycemia have been reported by world literature in patients with large malignant tumors without any signs of hyperinsulinemia (see Papaioannou, 1966; Shapot, 1972).

The basis for suspecting the "paraneoplastic" origin of the above pathologies is the impossibility of eliminating these syndromes by the

accepted and usually effective therapeutic measures (*diagnosis ex juvantibus*). A proper evaluation of the above complications offers prospects for revealing the malignant tumor during the preclinical period by the different forms of its pernicious action on the host. On the other hand, their "paraneoplastic" nature is in many cases demonstrated by the disappearance of the corresponding symptoms after removal of the discovered tumor.

We are still far from understanding the ways and means that connect the development of neoplasm with "paraneoplastic" phenomena. Only the first steps are being made in this direction. Elucidation of the mechanisms underlying these phenomena will apparently make it possible to enhance the resistance of the host to the tumor process by a scientifically substantiated correction of the disorders of homeostasis caused by the tumor and thereby in some measure to reduce mortality from cancer. The possibility of selectively sensitizing the tumor to the damaging factors employed in the clinic, viz., radiotherapy, chemotherapy, and hyperthermia, proved to be one of the helpful side effects of such studies. The achievements of selective "vulnerability" of the neoplasm makes it already possible to reduce the doses of these influences and safeguard against injury to the host tissues unaffected by the neoplasm (see Section IV).

In this article we are able to analyze only an insignificant part of the aforesaid "paraneoplastic" syndromes, but we deem it necessary to mention them in order to illustrate the multiformity and complexity of the effects exerted by the tumor on the host.

Moving on to the main subject of the article, we wish to note that we shall deal only with the most common of all malignant tumor effects on the host's homeostasis, omitting specific disorders caused by tumors of endocrine origin or by ectopic hormones produced by other tumors.

It is possible to distinguish at least two principal and interrelated groups of manifestations of systemic effect. One of them consists in changes in the metabolism and hormonal balance of the host caused by successful competition of the neoplasm with the normal tissues for vitally important metabolites and trophic factors. The other form may be defined as influence on distant tissues manifested by decreasing differentiation, changes in enzymic characteristics, diminution of the sensitivity of target tissues to the hormones that regulate their functions, and disturbance in the negative feedback systems which coordinate the activities of central and peripheral endocrine glands.

To designate "nontumor" syndromes encountered in oncologic patients, or the so-called complications of the main disease, the literature

makes use of most diverse terms, which hampers the analysis of corresponding phenomena, the determination of their relation to the tumor growth, and their rational classification.

A very popular term is "cancer intoxication." The idea of the general poisoning of the organism by products of tumor disintegration has no reasonable substantiation. Rapid disintegration of tumor tissue is a rare phenomenon, and cancer fever is now considered the result of autoimmune pathology or secondary infection, and nephritis is considered the manifestation of hyperuricemia.

No more felicitous is the term "cancer cachexia" since it has no concrete content. It does not indicate, for example, the specificity of cachexia for cancer. Moreover, the term produces an unjustified impression of an extreme degree of the oncologic patient's exhaustion. Actually, however, emaciation at the terminal stages of tumor development is not a frequent phenomenon at all. Shabad (1936) analyzed records of 932 autopsies performed in Leningrad on persons who had died of cancer of various sites and noted extreme general emaciation as the cause of death only in 11.8% of the cases. The emaciation was associated mainly with such tumor localizations as hindered the passage of food (cancer of the gullet, stomach, intestines). At the same time in some cancer loci the patients exhibited adiposis.

In the already mentioned study by Shabad (1936), 75% of the patients died not of a generalized tumor process, but of complications in the form of purulent infections.

The term "paraneoplasia" would seem to be more precise, but it, too, cannot be considered entirely adequate. The prefix "para" qualifies the phenomena as *concomitant* to the tumor process, whereas actually they are caused by it and it is for them the etiologic factor. Moreover, the term paraneoplastic is usually applied (see the Symposium "Paraneoplastic Syndromes," 1974) without any reason also to syndromes engendered not by all malignant neoplasms, but by only one of their specific groups, viz., tumors of endocrine origin uncontrollably producing corresponding hormones (insulinomas, pheochromocytomas, carcinomas of the adrenal cortex, etc.). We suggest that this category of phenomena should be designated as *hormonal tumor syndrome*, which is a narrower term than paraneoplasia.

If the tumor elaborates a product that is not characteristic of the homologous tissue, for example, adrenocorticotropic or parathyroid hormones in bronchogenic cancer, or erythropoietin in kidney cancer, it would be logical to add the word *ectopically* to the above term, the designation now reading *ectopically hormonal tumor syndrome.*

Of an entirely different nature is the feature common to all malignant

neoplasms, and unconnected with their elaboration of hormones, namely the ability to affect distant tissues and physiologic systems of the host, disordering its homeostasis, which in itself often leads to lethal results. In our opinion the most appropriate term for this property will be "systemic effect," which we are now going to use.

Cachexia may be recognized as a topical and not obligatory manifestation of the tumor's systemic effect on the host. Since cachexia may also be caused by other factors, it would be expedient to call it not "cancer cachexia," but "cachexia of the cancer patient."

There is an opposite aspect of the problem in question we are not able to touch upon in this paper. By this we mean induced alterations in the organism's homeostasis that either predispose or hamper tumor development ("anticarcinogenesis"). This topic has been competently covered by Kavetzky (1977) in his comprehensive monograph to which the reader is referred.

II. Competitive Relationships between the Tumor and the Host

A. Carbohydrate Metabolism

A notion of the tumor as a trap for glucose in the host put forward some years ago (Shapot, 1968, 1970, 1972, 1975) now seems to be reasonably well substantiated.

A tumor *in vivo* finds itself in a state of "glucose hunger" and is able additionally to consume and metabolize large quantities of glucose administered. An extremely high rate of glucose uptake by cancer cells as compared with a relatively slow glucose influx from the host underlies the above phenomenon.

As a result, low, sometimes undetectable levels of glucose are being maintained in the tumor itself and in surrounding medium (Gorozhanskaya and Shapot, 1964; Gullino *et al.*, 1964, 1967; Nakamura and Hosoda, 1968; Shapot 1972, 1975).

Recently additional data supporting this view have been obtained. Using a highly sensitive modification of the glucose oxidase method, permitting the determination of as low a glucose concentration as 0.0005 mg/sample, Shapot *et al.* (1976) could not detect even traces of glucose in highly malignant mouse Guelshtein 22a, 61, and 60 hepatomas. As to the slow growing Guelshtein 48 hepatoma, in some instances traces of glucose were found.

The above regularity does not seem to be confined to transplantable tumors but holds for human malignancy as well. In all malignant tumors

(surgically removed and thereafter immediately placed in 0.02 *M* NaF to prevent glycolysis) of stomach ($n = 19$), mammary ($n = 15$), uterus ($n = 18$), kidney ($n = 8$), ovary ($n = 3$) examined, no glucose was found. In benign human blastomas, glucose levels ranged from 0.08 to 0.22 mg/g.

In tissues of healthy subjects (victims of accident) [lung, stomach, kidney ($n = 10$, each), mammary, gland, heart, uterus, ovary ($n = 3$, each)], the content of glucose ranged from 0.17 to 1.24 mg/gm[1] (Shapot *et al.*, 1977).

It is worth mentioning here that in human malignant tumors of stomach, lung, uterus, and ovary the hexokinase, a key enzyme of glycolysis, was shown to have K_m about 10 times lower (10^{-6} *M*) than that of the homologous human normal tissues (Monakhov *et al.*, 1978). It is likely that a high affinity of hexokinase for glucose endows the malignant neoplasm with the advantage to metabolize glucose at such negligible concentrations.

Undetectable glucose levels in the tumor tissue creates an enormous glucose concentration gradient between the arterial blood and tumor—80 mg/100 ml versus zero! Such a gradient favors successful competitior of the tumor with the host tissues for glucose and ensures a permanent hypoglycemic strain on the host.

Severe hypoglycemia found in animals carrying ascites Ehrlich carcinoma and Brown-Pierce carcinoma was already described earlier (Blinov *et al.*, 1974; Shapot and Blinov, 1974; Shapot and Blinov, 1975; Shapot, 1975). Numerous data from the literature as to profound hypoglycemia in cancer patients with large (from 900 gm to 18 kg) tumors of nonendocrine origin were also listed (Shapot, 1972).

Analysis of the above observations led us to the conclusion that the hypoglycemia in question was induced by the tumor avidly consuming glucose in case compensatory mechanisms of the host failed to counterbalance this tumor activity. Some other authors are of the same opinion (Papaioannou, 1966; Carey *et al.*, 1966; Nissau *et al.*, 1968; Jacob *et al.*, 1969; Chowdhury and Bleicher, 1973; Marks *et al.*, 1974).

Direct measurements attest to an extremely high rate of glucose uptake by the tumor. Utilization of glucose by a woman with intensively metastasizing leiomyosarcoma was found to be 12 mg/kg/min, i.e., 4 to 12 times as high as normal (Carey *et al.*, 1966).

However, many clinicians still maintain that hypoglycemia in cancer patients with extrapancreatic tumors is due to hyperinsulinemia or to an excess of insulinlike peptides (NSILA) in the blood, presumably pro-

[1] The above values are of course, underestimated, since corpses lay several hours prior to the time samples were taken, and intensive glycolysis could proceed during that time.

duced by the neoplasm. We believe that it is time to give up such a view. Sometimes hypoglycemia in tumor-bearing animals and cancer patients develops concomitantly with normal or even decreased blood insulin levels.

As for animals, we have shown (Shapot and Blinov, 1975) that hypofunction of insulin-producing apparatus was provoked by a hypoglycemic status of the host (feedback effect). Rabbits carrying Brown-Pierce carcinoma characterized by a marked hypoglycemia (40 mg/100 ml) display after glucose load a flattened glycemic curve typical of latent diabetes, A similar phenomenon in cancer patients was noted by many authors. More spectacularly, functional insulin deficiency manifests itself when a repeated (double) glucose load was applied to rabbits with Brown-Pierce carcinoma whose level of immunoreactive insulin in the blood was found to be close to normal (15 ± 2.0 versus 16 ± 3 μU/ml in controls).

The second injection of glucose to control animals, 30 minutes after the first one, was accompanied by a decline of the hyperglycemic curve, whereas in rabbits carrying Brown-Pierce carcinoma the second glucose load provoked a sharp jump of the glycemic curve. When, however, the rabbits were "saturated" with glucose by infusion 30 days running from the very moment of tumor implantation, a relative hyperglycemia could be maintained, and the second glucose load was followed by a more drastic decline of blood sugar levels than that observed with control groups. Thus, hypoglycemia in tumor-bearing animals being prevented, sufficient insulin was released to effectively eliminate an excess of the blood glucose.

In mice with Ehrlich carcinoma who also developed severe hypoglycemia (50 mg/100 ml versus 90 mg/100 ml in intact mice), serum insulin levels sharply decreased (from 16 ± 2.0 down to 11 ± 1.5 μU/ml) as the mass of ascites cancer cells enlarged (Blinov, 1974). In rats carrying Zajdela hepatoma with only a slight hypoglycemia, the serum insulin concentrations was found somewhat diminished. Thus the above observations do not support the idea that hypoglycemia in tumor hosts is provoked by the action of an excess of insulin circulating in the blood.

Marks *et al.* (1974) examined three patient with (1) Hodgkins disease, (2) an anaplastic large metastasizing melanoma, and (3) fibrosarcoma of enormous size. All patients mentioned suffered from severe hypoglycemia. A continued infusion of 10% glucose was necessary to prevent hypoglycemia in patient 2, the fasting serum insulin levels in all three patients being barely detectable. Two patients, however, had elevated serum NSILA levels.

Two weeks after surgical removal of the tumor, the blood sugar in female patient 3 rose from 44 mg/100 ml to 96 mg/100 ml, but the serum

NSILA dropped from 850 to 250 μU/ml. However, 17 months later her serum NSILA was found elevated again with no sign of hypoglycemia. On incubation of the melanoma removed from patient 3 the tumor released neither immunoreactive insulin nor NSILA into the medium. The above authors, as we do, believe that hypoglycemia induced by the growing tumor is responsible for hypofunction of the host's insulin-producing apparatus.

Why don't the majority of tumor-bearing animals and cancer patients develop hypoglycemia? It is obvious that glycogenolysis and gluconeogenesis as compensatory mechanisms must be involved in overcoming the hypoglycemic pressure exerted by the tumor on the host.

There is a growing body of evidence to indicate that the liver and the muscle of tumor-bearing animals lose their glycogen as the tumor is progressing. This was the case with the liver of rats carrying Walker 256 carcinoma and of mice with Ehrlich ascites carcinoma (Granzov and Beheim, 1972). The content of liver glycogen in mice with Guelshtein 22a hepatoma and in rats with Zajdela hepatoma was found to be as low as 13% to 32% of that of controls (Mishineva *et al.*, 1973). Only 5% and 10% of the original content of glycogen in rabbit liver and muscle, respectively, was left by day 20 after implantation of Brown-Pierce carcinoma, the dissemination of tumor nodes in the host being in contrast drastically increased: by day 10, 20, and 30 up to 25.1 ± 2.4; 58 ± 8.8, and 93.8 ± 4.0 (in arbitary units), respectively (Shapot and Blinov, 1975).

Potential capacity of mouse, rat, and rabbit liver to synthesize glycogen during tumor growth was not found to be impaired (Pattillo, 1971; Mishineva *et al.*, 1973; Shapot, 1975). Hence the only reason for liver glycogen depletion must be its intensive mobilization to meet the glucose requirements of both the tumor and the host.

However, depletion of liver and muscle glycogen in rabbits with Brown-Pierce carcinoma did not prevent hypoglycemia attaining 38 mg/100 ml by day 30 after tumor implantation (Shapot and Blinov, 1974). Therefore mobilization of glycogen storage may play only a minor role in maintaining normoglycemia in the tumor host. But it is possible that depletion of both liver and muscle glycogen serves as a trigger to intensify the main compensatory mechanism, namely, glyconeogenesis from noncarbohydrate compounds (see below).

There are some observations that seem to support such a proposition (Marks *et al.*, 1974). A patient with melanoma developed severe hypoglycemia. On autopsy, his liver with no sign of metastases was rich in glycogen. Thus in the above case there was a correlation between insufficient gluconeogenesis and the loss by the liver of the capacity to mobilize glycogen.

However, in some other instances the above correlation did not exist. Gluconeogenesis was stimulated in rats carrying Zajdela hepatoma, although in their liver a large amount of glycogen was found (our observations). Similar findings were reported by Nisselbaum (1972). No depletion of liver glycogen occurred in rats with Morris 5123 hepatoma even after 24 hours starvation, and their blood sugar levels remained close to normal values. Probably phosphorylase of these animals could not be converted to the active "a" form, as was the case with certain hepatomas (Sato and Tsuiki, 1972).

Gluconeogenesis in the tumor host had not been studied systematically and directly as a process. It was assessed sometimes in cancer patients indirectly by determining the activation of the Cori cycle (see e.g., Holroyde *et al.*, 1975).

We undertook a systematic study of gluconeogenesis from noncarbohydrate compounds in rabbits, rats, and mice with various types of tumors. Labeled amino acids ^{14}C-tyrosine, ^{14}C-glycine, ^{14}-glutamic acid, and ^{14}C-alanine were used as glucose precursors. We focused on glucogenic amino acids since extensive protein catabolism occurs in the tumor host favoring gluconeogenesis from these sources.

Newly formed radioactive glucose was isolated either by paper chromatography or by a highly specific method—chromatography on an ion-exchange column using glucose oxidase (Friedman *et al.*, 1967).

Gluconeogenesis was studied in liver and kidney of tumor-bearing and intact animals. The liver is known to maintain normoglycemia altering the rate of gluconeogenesis in correspondence with fluctuations in the composition of the body's inner medium (Exton, 1972; Eisenstein, 1973). The kidney is also involved in this process for the inhibition of gluconeogenesis in both kidneys results in hypoglycemia within an hour (Cahill, 1970).

According to Felig (1975), alanine comprises about half of all amino acids taken up by human liver and serves as the main precursor of glucose formed from products of muscle protein catabolism. A similar phenomenon was observed in animals (Felig, 1975). Preferential conversion of the carbon skeleton of alanine to glucose when compared with gluconeogenesis from other glucogenic amino acids was fully confirmed in our experiments on animals.

It turned out that all animals studied fell into two distinct groups, depending on the type of tumor they had. In mice with Crocker carcinoma, Ca-755, Guelshtein solid and ascites hepatomas and in rats with Zajdela hepatoma, normoglycemia was maintained until death, and only slight hypoglycemia in Zajdela hepatoma-bearing rats was sometimes observed.

Animals of the second group (mice with Ehrlich ascites carcinoma and rabbits with Brown-Pierce carcinoma) developed severe hypoglycemia.

A direct correlation between the normoglycemia and stimulated gluconeogenesis in tumor-bearing animals was established (Shapot and Blinov, 1974; Blinov, 1974; Shapot, 1975; Blinov *et al.*, 1975).

In the first group endogenous formation of glucose from labeled amino acids was found to be elevated manyfold, especially in the case of large tumors. For instance, in rats with Zajdela hepatoma by day 5 after implantation the content of the radioactive glucose in the blood, liver, and kidney increased 2-fold, in mice with Guelshtein solid hepatoma by day 20 after implantation—3.5-, 5.5-, and 6-fold, respectively.

As for mice with Ehrlich ascites carcinoma characterized by growing hypoglycemia, their gluconeogenesis was markedly inhibited by day 4 after implantation (Table I).

In rabbits with Brown-Pierce carcinoma, who also developed hypoglycemia, gluconeogenesis was inhibited only in the kidney cortex; in liver it was stimulated six-fold. However, the concentration of radioactive glucose in the blood was not elevated. Hence in this instance a compensatory intensification of liver gluconeogenesis was not sufficient to counterbalance the loss of glucose trapped by the tumor disseminated throughout the host tissues.

The next question to be answered was whether it was possible to unmask the tendency toward hypoglycemia even in cases where normal sugar levels were maintained, as in mice (CBA × C57BL) F_1 with Ca-

TABLE I

GLUCONEOGENESIS[a] IN MICE WITH EHRLICH ASCITES CARCINOMA

Mice		Tissues		
		Blood	Liver	Kidney
From ^{14}C-tyrosine:				
Control		1,350 ± 133	1,070 ± 130	730 ± 86
Carrying Ehrlich carcinoma	4 days	290 ± 32	1,150 ± 135	—
	8 days	1,320 ± 200	1,330 ± 205	1,230 ± 270
From ^{14}C-glycine:				
Control		15,600 ± 1,600	22,140 ± 2,210	12,550 ± 2,920
Carrying Ehrlich carcinoma	4 days	7,680 ± 640	14,820 ± 1,600	10,400 ± 1,260
	8 days	8,540 ± 1,020	16,010 ± 2,600	10,050 ± 990

[a] Gluconeogenesis expressed as ^{14}C-glucose (cpm/ml or cpm/mg tissue) formed.

755. Here we made use of the approach that included a continuous "saturation" of the host with glucose from the very moment of tumor implantation and subsequent cessation of glucose injection after a certain period.

Both intact and tumor-bearing (CBA × C57BL) F_1 mice were injected with 10 mg/gm glucose for 10 days, and then the extra glucose supply was discontinued and the animals were left on the standard diet. Intact and tumor-bearing mice of the same line, which did not receive extra glucose, were under observation as well.

As could be expected, the rate of liver and especially kidney gluconeogenesis declined as a result of glucose injections in both control and tumor-bearing animals. However, the difference between them was revealed after the extra glucose supply ceased. In healthy mice the rate of glucose formation from ^{14}C-tyrosine increased somewhat, but the initial level was not obtained within the next 10 days. In contrast, gluconeogenesis in tumor-bearing mice under the same conditions was sharply accelerated, especially in the kidney cortex, and highly exceeded the normal level. As to the tumor-bearing mice that did not receive glucose, gluconeogenesis in their kidney increased continuously after implantation (Fig. 1). These data support the idea that normoglycemia in mice carrying Ca-755 is maintained owing to elevated gluconeogenesis.

In spite of two-, four-, and sevenfold stimulation of liver and kidney gluconeogenesis, the concentration of ^{14}C-glucose in the blood is in-

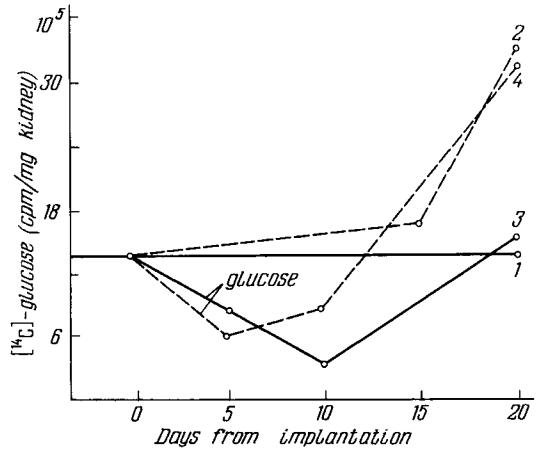

FIG. 1. Gluconeogenesis in the adrenal cortex of control (1, 3) and tumor-bearing (2, 4) mice. 3 and 4 were injected with glucose (10 mg/gm weight) twice a day for the first 10 days.

creased no more than by 50%, this fact presumably reflecting an intensive utilization of glucose by the tumor.

In the other experiments the dynamics of glycogen accumulation in liver as well as the rate of its synthesis from ^{14}C-tyrosine in this organ were studied. From Fig. 2 one sees depletion of liver glycogen in non-starving mice by day 20 after tumor implantation; its content decreased more than 90%-fold.

As a result of a continuous injection of glucose, liver glycogen in both intact and tumor-bearing mice increased manyfold, but after extra glucose supply ceased the content of liver glycogen in control mice gradually declined, never reaching the initial level; whereas in tumor-bearing animals depletion of liver glycogen proceeds very rapidly to the level characteristic of tumor-bearing mice that had no glucose injections.

The most plausible explanation for the above observations is an intensive mobilization of liver glycogen to counterbalance the tendency toward hypoglycemia induced by the tumor.

Figure 3 shows a stimulation of the synthesis of ^{14}C-liver glycogen in tumor-bearing mice under usual conditions. Continuous injections of glucose inhibited liver gluconeogenesis, but cessation of extra glucose supply from day 11 onward was followed by a sharp stimulation of this process in the case of tumor-bearing mice only, whereas in intact animals the specific radioactivity of the newly formed glycogen from ^{14}C-tyrosine increased slowly and within 10 days remained lower than the initial values.

Thus the results described reveal a direct correlation between the

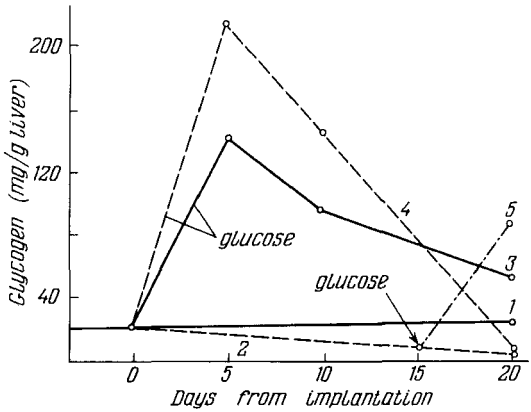

FIG. 2. Accumulation and depletion of liver glycogen in control (1, 3) and tumor-bearing (2, 4, 5) mice. 3 and 4 were injected with glucose twice a day for the first 10 days, 5, for the last 5 days.

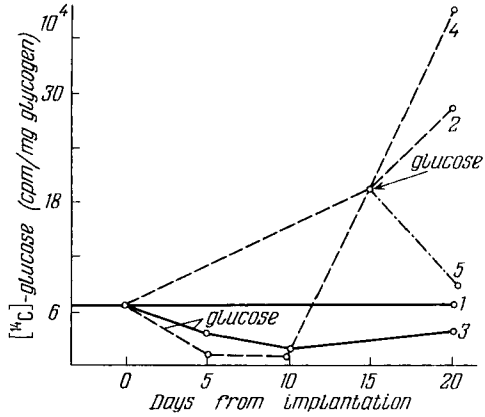

FIG. 3. ^{14}C-glycogen formation from U-^{14}C-tyrosine in the liver of control (1, 3) and tumor-bearing (3, 4, 5) mice. 3 and 4 were injected with glucose twice a day for the first 10 days; 5, for the last 5 days.

depletion of host liver glycogen and a drastic acceleration of liver glyconeogenesis induced by the avid uptake of glucose by the tumor (Blinov and Shapot, 1974b; Shapot, 1976).

What are the factors responsible for the stimulation of gluconeogenesis in the host? Gluconeogenesis is under multihormonal control. Insulin acts as an inhibitor; glucagon, epinephrine, growth hormone, and glucocorticords accelerate endogenous formation of glucose.

We do not know of any data indicating elevated levels of the blood glucagon. The same holds for epinephrine, although the possibility of its hypersecretion is consistent with the phenomenon of a stimulated lipolysis in adipose tissues and muscle described for the tumor's host.

As for glucocorticoids, there are many observations as to their elevated levels both in cancer patients (Saez, 1971, 1974), and tumor-bearing animals (Samundjan, 1973).

Glucocorticoids induce hyperglycemia in rats (Herrmann and Staib, 1969), cows (Heitzman *et al.*, 1971), and man (Kelly, 1959; Egorova, 1965) and raise the activity of key enzymes of gluconeogenesis (Weber, 1968; Knox and Sharma, 1968; Mertvetzov, 1969).

We believe that hyperfunction of the adrenal cortex may play an important role in the intensification of glyconeogenesis as a response to hypoglycemic strain of the tumor on the host.

In our experiments one injection of cortisol into intact mice starved for 24 hours induced a gradual elevation of the blood sugar especially pronounced by the 24th hour. A similar phenomenon has been noted

with respect to liver glycogen. Its content increased sevenfold within 24 hours after cortisol injection. Formation of glycogen from labeled amino acids was also accelerated five- to sixfold (Blinov *et al.*, 1975).

Here we would like to draw attention to the fact that cortisol injected in the same doses to mice with Ehrlich carcinoma, which developed severe hypoglycemia, failed to stimulate gluconeogenesis, unlike its normal effect exerted on mice with Crocker sarcoma, which were able to maintain normoglycemia. Daily injection of large doses of cortisol (5 mg) for 8 days were needed to raise the blood sugar level in mice with Ehrlich carcinoma, this observation indicating an elevated threshold of sensitivity to the hormone.

It is appropriate to mention a report on two cases by Nissau *et al.* (1968). Cancer patients with large tumors, reticulosarcoma and lymphosarcoma, developed pronounced hypoglycemia. Injection of glucocorticoids or epinephrine failed to raise their blood sugar levels. The authors suspected impairment of gluconeogenesis in both patients which was not able to counterbalance an excessive uptake of glucose by the tumor (studied *in vitro* after its surgical removal).

All findings described above support our view that normoglycemia in the tumor's host is ensured by stimulated gluconeogenesis from noncarbohydrate sources, its impairment resulting in hypoglycemia.

B. Nitrogen Metabolism

The tumor is a trap for nitrogen, a concise and expressive term coined by Mider (1951, 1953) as a result of his excellent studies. However, Mischenko (1940) seemed to be the first to discover the capacity of the tumor to take up nitrogen of the host's tissue proteins. The experiments were performed on transplantable rat and chicken tumors, but his observations applied to cancer patients as well. Mischenko inferred that the tumor is growing at the expense of assimilation of products of host's muscle protein breakdown. He wrote that the tumor is able to consume both exogenous and endogenous nitrogen compounds and ensures its endogenous nutrition inducing an "intertissue exchange." Mischenko's experiments demonstrated that even during starvation the host supplies the tumor with building blocks and that cachexia may be caused by an intensified hydrolysis of host tissues.

Mider's and Mischenko's idea was confirmed by LePage *et al.* (1952). Starving rats carrying a rapidly growing Flexner carcinoma lost within 5 days 31% of their weight and 39% of liver protein. In spite of this the weight of the carcinoma markedly increased; its growth rate being only

half as high as in similar animals kept on a protein-rich diet. Rat proteins and nucleic acids, prelabeled with ^{14}C-glycine, after tumor implantation lost their label very rapidly, whereas the specific radioactivity of tumor proteins and nucleic acids sharply increased.

It is quite obvious that the tumor somehow enhances protein catabolism of the host tissues and may at the same time hinder protein synthesis in them.

The data of Lundholm (1975) and Lundholm *et al.* (1976) support this view. In 43 patients with various sites of tumor (esophageal, gastric, hepatic, pancreatic, colon, rectal, renal carcinomas), the above authors found a significant elevation of the cathepsin D activity in muscle lysosomes, enhancement of muscle protein breakdown, and a slower than normal protein synthesis as revealed by measuring incorporation of labeled leucine performed *in vitro* (biopsy). It was possible to stimulate the incorporation rate of leucine into soluble protein in cancer patient's muscle fibers by increasing the normal human plasma amino acid level 10-fold in the incubation medium. The degree of stimulation was equal to that observed in control specimens. According to Lundholm (1975), the above effect distinguished cancer disease from malnutrition.

In Blinov's experiments (1974) in rabbits with Brown-Pierce carcinoma the total nitrogen content sharply decreased in the heart by day 10, and in skeletal muscle by day 20 after implantation. The host finds itself under subcaloric conditions, therefore preferential breakdown of muscle proteins to meet the requirements of visceral organs and the tumor might be expected.

Numerous nitrogen balance studies both with tumor-bearing animals and cancer patients failed to unambiguously answer the question. Hence warranted doubts were expressed as to the validity of such an approach (Costa, 1977; Blackburn *et al.*, 1977). Indeed, in our experiments on rabbits with Brown-Pierce carcinoma a pronounced negative nitrogen balance within 13 to 18 days after implantation was observed. In the terminal stage quantities of nitrogen excreted within 2 days were as high as about 1.5 gm. In this case the dissemination of the tumor throughout the host tissue attained such a degree that it was not able to trap all the nitrogen released by the host.

In contrast, in rat 45 sarcoma, which grew locally, during the whole period after implantation, an apparent positive nitrogen balance was found to be maintained in the host. The weight of the animal carcass (less 15 to 49 gm neoplasm) was reduced on the average by 8 gm. Hence exogenous, dietary nitrogen as well as endogenous nitrogen seemed to be consumed by the growing tumor (Shapot and Blinov, 1975). Similar observations on cancer patients were reported by Blackburn *et al.* (1977).

The most physiologically significant tissue losses were from the viscera, indicated by the low albumin, transferrin, and total lymphocyte count accompanied by the marked impairment of cell-mediated immunity.

The reason for stimulated catabolism of nitrogen biopolymers in the tumor host remains unclear. One may assume that both starvation and certain hormonal imbalance, in part hypersecretion of glucocorticoids, are involved.

Hypertrophy of the adrenal cortex of the tumor host with the eventual dystrophy of the gland by the terminal stage of tumor growth drew the attention of many authors long ago (Begg, 1958). A hypersecretion of glucocorticoids in cancer patients (Saez, 1971; 1974) and tumor-bearing animals (Samundjan, 1973) was also reported.

According to our observations made with Bunatyan (Shapot and Blinov, 1975), the concentration of the total 11-oxycorticosteroids as well as of biologically active corticosterone in the blood of rabbits with Brown-Pierce carcinoma by days 20 and 30 after implantation was significantly elevated.

There are reasons to believe that hypersecretion of glucocorticoids may cause stimulation of protein breakdown, especially in the tumor's host. Blinov (1974) has shown that cortisol administered to rabbits with Brown-Pierce carcinoma additionally stimulated catabolism of nitrogen polymers. The content of the total nitrogen in urine increased 16%, NH_3 100%, and creatin 110% as compared with tumor-bearing rabbits that received no hormone. Moreover, cortisol exerted on tumor-bearing rabbits a more pronounced effect than on healthy ones. Excretion of NH_3 and creatine by the former was three times higher than that by the latter.

Jewell and Hunter (1971) reported that adrenalectomy prevented the stimulation of albumin catabolism in rats with Walker 256 carcinoma. Thus there is an apparent similarity in the ability of glucocorticoids and the tumor to enhance protein catabolism in the body.

The following question arises: In what way does the tumor induce a hypersecretion of glucocorticoids in the host? We have already described the role played by stimulated gluconeogenesis in counterbalancing hypoglycemic pressure exerted by the tumor.

Glucocorticoids are known to enhance gluconeogenesis, and it is likely that their hyperproduction in the host is just a response to the tendency toward hypoglycemia induced by the tumor. If true, "saturation" of the host with glucose would reduce catabolic processes.

Indeed, administration of glucose was shown to reduce the losses of nitrogen by surgical noncancer patients (Abbott *et al.*, 1959; Holden *et al.*, 1957) and starving animals (Strautmans and Schmidt, 1966). A high-carbohydrate diet tended to normalize previously elevated levels of free

amino acids in the host's blood to the same degree as did removal of the tumor (Mustea, 1971). Food enriched in carbohydrates prevented the loss of nitrogen in the brain of mice carrying MFS fibrosarcoma (Mallick *et al.*, 1968).

Blinov (1974) (see also Shapot and Blinov, 1975) administered 2 gm/kg 40% glucose daily to rabbits with Brown-Pierce carcinoma. As a result their blood total nitrogen, urea, glutamine, nitrogen of free amino acids, creatine, and creatinine sharply decreased as compared with carcinoma-bearing rabbits that did not receive glucose. The content of the total nitrogen in the spleen and myocardium increased as did creatine in the latter tissue; the level of creatinine was reduced.

Thus, a prolonged "saturation" of the host with glucose obviously alleviates catabolic effects of the tumor. In this connection it is worth mentioning again that excess of administered glucose inhibits gluconeogenesis (see Section II,A).

Factors that favor the uptake of amino acids as products of the host's tissue protein catabolism by the tumor remain unknown. Some speculations, however, may be made concerning glutamine. Glutamine is known to serve as one of the most important precursors of the synthesis of tumor protein and purine nucleotides. The content of glutamine in tumor cells, however, was found to be negligible (Roberts *et al.*, 1971) owing to its extremely rapid assimilation and a low capacity of the tumor to synthesize glutamine (Schreck *et al.*, 1973). In the host tissues, glutamine levels gradually decreased and sometimes, as in the case of rapidly growing Walker 256 carcinoma and Novikoff hepatoma, dropped to barely detectable values (Wu and Morris, 1970).

Similar results were obtained (Shapot and Blinov, 1975) with rabbits with Brown-Pierce carcinoma. From day 10 after implantation onward the content of glutamine in rabbit liver, heart, and skeletal muscle (but not in kidneys) decreased, while that in the blood increased. The latter fact suggested the release of glutamine from host tissues.

Proceeding from the observations described above, one may propose that a preferential transfer of blood glutamine to the tumor is conditioned by a high gradient between its concentration in the arterial blood and that in the tumor, as it occurs with glucose (see Section II,A).

Dystrophy of vital organs may to a certain degree be conditioned not only by enhanced catabolism of their proteins, but by a reduced rate of protein synthesis also. A fraction of glucogenic amino acids would inevitably be channeled to the carbohydrate metabolic pathway when gluconeogenesis was stimulated in the tumor host. Thereby this fraction would not be available any longer for the participation in polypeptide chain formation.

Another reason for a hindered protein synthesis in the host may be impairment or rearrangement of the protein-synthesizing apparatus. Clark and Goodlad (1975) have described a certain deficiency in ribosomes of skeletal muscle as a result of the pernicious effect of Walker 256 carcinoma growing in rats. It is widely known that both in cancer patients and tumor-bearing animals dystrophy of skeletal muscle occurs very often. Clark and Goodlad (1975) studied ribosomes of gastrocnemius very thoroughly. Their protein-synthesizing capacity was only half as high as that of corresponding muscle of intact rats. Hybrid ribosomes were prepared composed of the large ribosomal subunit of gastrocnemius from the tumor host and the small unit from the normal muscle and vice versa. The defect was found to be localized in the small subunit after the initiation of polypeptide chain synthesis.

There are other data indicating a rearrangement of the protein-synthesizing apparatus in the liver of the tumor host, for example, a shift in the proportion of membrane-bound and free ribosomes in favor of the latter. In normal rat liver, according to our observations, the membrane-bound to free ribosomes ratio is 3.0 ± 0.25. This ratio is a reflection of a high specialization of hepatocytes since membrane-bound ribosomes, unlike free, synthesize preferentially extracellular proteins subsequently transferred to the circulation. According to Yap *et al.* (1977), 98% of mRNA coding for serum albumin is associated with membrane-bound polyribosomes.

In hepatomas the opposite picture can be seen: at the early stages of growth the relative proportion of membrane-bound ribosomes is reduced and the membrane-bound to free ribosomes ratio eventually drops to 0.5 ± 0.14.

From day 3 after implantation of Zajdela hepatoma onward the relative proportion of membrane-bound ribosomes in rat liver starts reducing and by day 6 the above coefficient reaches the value of 0.63 ± 0.26, very close to that characteristic of the tumor itself. Special measures are taken to collect all cytoplasmic membrane-bound ribosomes including those sedimenting with mitochondria and nuclei (Pushkina *et al.*, 1976).

One of the possible interpretations of the phenomenon described would be that it is a defense reaction of the host against the action of the tumor as a trap for nitrogen—the protein-synthesizing apparatus is reformed to ensure the synthesis of intracellular, structural proteins. Indeed, the pool of free ribosomes was found to be enlarged on malnutrition (Ekren and Vatrin, 1972). We made attempts to verify the above observation and let intact rats starve for 48 hours with no limitation in water supply. The proportion of free ribosomes in their liver was reduced from 3 ± 0.25 to 0.78–1.3.

However, forced nutrition of rats with Zajdela hepatoma within the 2 last days when anorexia sets on (infusion of glucose, interlipid and protein hydrolyzate) prevented the reduction of the membrane-bound to free ribosomes ratio only partially; it did not rise higher than 1.

Hence, the effect of the tumor on the host liver ribosomes cannot be explained only by starvation.

As for rat regenerating liver (48 hours after partial hepatectomy) the opposite picture could be seen—the proportion of membrane-bound ribosomes rose up to 83%–85% of total ribosome pool.

In this connection one more example of the functional reformation of protein-synthesizing machinery in the host liver can be mentioned (Shapot and Berdinskich, 1975; Shapot, 1975). It is well known that tumor growth both in humans and animals is often accompanied with hypoalbunemia. The reason for this may be at least twofold, a result of stimulated albumin catabolism (Jewell and Hunter, 1971) or hampered synthesis of this protein, or both. Specific immunoprecipitation of polyribosomes with antibodies against serum albumin followed by ultracentrifugation of the precipitate on sucrose concentration gradient allowed us to demonstrate in the case of animals with Schwetz leukemia and Zajdela hepatoma that in their livers the fraction of polyribosomes involved in the formation of albumin polypeptide chains was sharply reduced (Fig. 4), the total content of polyribosomes remaining unchanged. The Zajdela hepatoma completely lost the capacity to synthesize serum albumin.

The phenomena described above are regarded as manifestations of dysdifferentiation of the host's vital organs which synthesize proteins

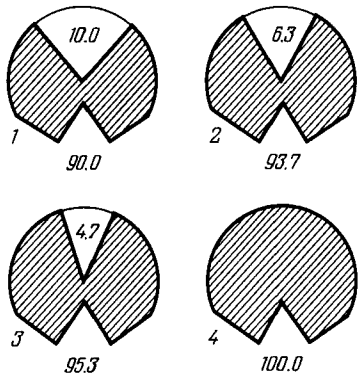

FIG. 4. Proportion (in percent of total) of rat liver and hepatoma polyribosomes synthesizing serum albumin: 1, control: 2, animals with Schwetz leukemia: with Zajdela hepatoma; 3, 4, Zajdela hepatoma cells.

needed for the whole body at a reduced rate. Both in trauma and tumor growth, the synthesis of secreted proteins such as transferrin, lipoproteins, and serum albumin decreased (Blackburn *et al.*, 1977).

Our knowledge of nucleic acid metabolism in the tumor host is scarce. Here we report some recent data on the successful competition of the tumor with the host lymphoid tissues for the precursors of nucleic acid, pyrimidine nucleotides.

The common pyrimidine precursor of RNA—uridine monophosphate (UMP)—is synthesized through two different pathways: (a) *de novo*, from CO_2, NH_3, and aspartic acid, with the formation of an intermediate compound, orotic acid; and (b) a "salvage" route, including the reaction of uracil with ribosylpyrophosphate, leading to uridine. Proportions of these two pathways vary depending on the type of tissue, but the "salvage" one prevails in rapidly growing malignant tumors.

Comparative study of the proportions of the above pathways as a reflection of the effect of the tumor on the host proved very informative. According to our previous observations with Vornovitskaya (see Shapot, 1975), rat liver utilizes mainly orotic acid as a precursor of RNA pyrimidine nucleotides, the incorporation of uridine into RNA being 22-fold lower. In regenerating liver this difference in favor of orotic acid rises to about 60. As for rat Zajdela hepatoma, the opposite picture is seen. Uridine serves as a predominant precursor of RNA pyrimidine nucleotides, its incorporation being 10 times that of orotic acid.

The most spectacular results were obtained (Vornovitskaya *et al.*, 1979) with C3HA mice carrying rapidly growing Guelshtein 22a hepatoma, which like Zajdela hepatoma utilizes mainly uridine. In mouse liver, unlike rat liver, both orotic acid and uridine were involved in RNA synthesis equally. In contrast, in the mouse spleen and thymus, the preferential precursor of RNA pyrimidine nucleotides turned out to be uridine. The incorporation ratio of ^{14}C-orotic acid to ^{14}C-uridine into RNA in the above lymphoid organs was 0.21–0.22, whereas in 22a hepatoma it was only 0.05.

We have studied the changes in this ratio in the process of tumor growth (Fig. 5). In the liver and spleen orotic acid incorporation remained practically unchanged within day 1 to day 8 after implantation, in thymus being even somewhat higher; whereas the incorporation of uridine sharply decreased, particularly in the spleen. It dropped after 24 hours after implantation to as low as 13%, and on the fifth and eighth days to 4% of the initial value (Fig. 6). Hence the tumor intercepts for its own needs the precursor that is required first, omitting orotic acid which is utilized poorly. It is obvious that such a pumping of uridine would affect lymphoid organs first, while the liver can effectively utilize orotic acid.

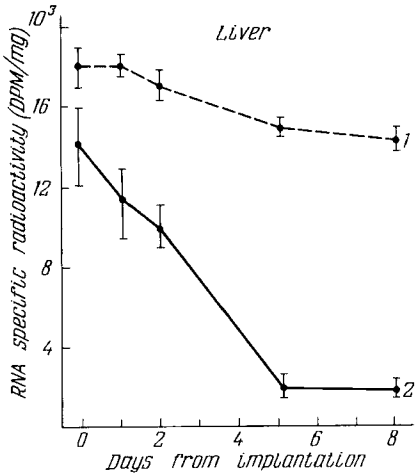

FIG. 5. Incorporation of ^{14}C-orotic acid (1) and ^{14}C-uridine (2) into liver RNA of hepatoma-bearing mice.

The phenomenon of successful competition of the tumor with the host for thymidine as a precursor of DNA was demonstrated by us on C3HA mice with Guelshtein hepatomas as well (Gerstein *et al.*, 1978). In the acid-soluble fraction of 22a hepatoma, on the fifth day after implantation, as early as 5 minutes after ^{14}C-thymidine administration, the label was found to be very high and then gradually declined within 55 minutes, obviously being utilized in DNA synthesis since the label in DNA rose in parallel (Fig. 7). An intensive incorporation of the labeled exogenous

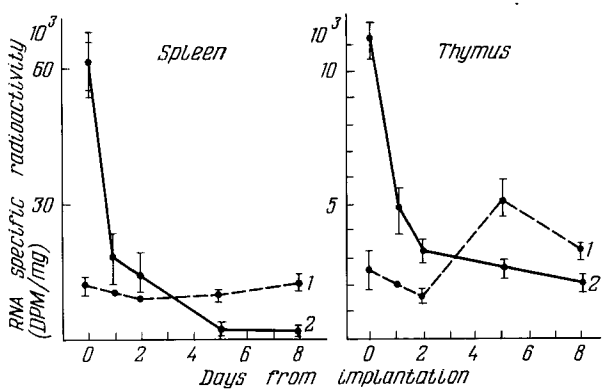

FIG. 6. Incorporation of ^{14}C-orotic acid (1) and ^{14}C-uridine in spleen and thymus RNA of hepatoma-bearing mice.

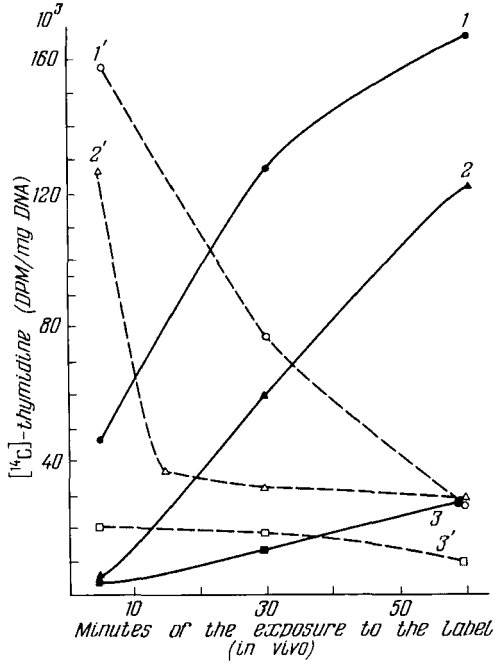

FIG. 7. Incorporation of ^{14}C-thymidine into mouse hepatoma DNA (1, 2, 3) and acid-soluble fraction (1', 2', 3'): 1 and 1', 22a hepatoma on the fifth day of the implantation; 2 and 2', 22a hepatoma on the eighth day, 3 and 3', 48 hepatoma (1–1.5 month growth).

thymidine into DNA of rapidly growing Morris hepatomas was described earlier by the Weber group (Ferdinandis *et al.*, 1971). On the eighth day after implantation close to the terminal period, the initial (5 minuntes) level of the ^{14}C-thymidine label in the acid-soluble fraction was markedly reduced as well as the rate of its incorporation into DNA. In slow growing Guelshtein 48 hepatoma both of the above indices were low.

The kinetics of the incorporation of ^{14}C-thymidine into DNA and the ^{14}C-label in the acid-soluble fraction of the spleen of intact and 22a hepatoma-bearing mice was also studied. On the second day from implantation a reduced radioactivity, calculated on the basis of mg DNA, could be observed within 5 minutes. On the fifth day, the period of rapid tumor growth, both the initial value of the label in the acid-soluble fraction and the rate of ^{14}C-thymidine incorporation into DNA were decreased about 60% (Fig. 8). It is likely that rapidly growing 22a hepatoma intercepts exogenous thymidine for its own DNA synthesis, interfering with the influx of thymidine to even rapidly proliferating host tissues.

Slow growing 48 hepatoma did not alter significantly thymidine incorporation into DNA and its influx into the acid-soluble fraction in any of the host tissues studied. The same holds for rat Zajdela hepatoma.

Suppression of DNA synthesis in the spleen of 22a hepatoma-bearing mice seems to be a result not just of a competition of the tumor for the precursor, but of other factors as well. We noted certain alterations in the distribution of the ^{14}C-label in the spleen acid-soluble fraction between thymidine (along with products of its catabolism), TMP, and TDP + TTP (Table II). From the second day onward a specific distribution of the label in the spleen was shifted in favor of nonphosphorylated compounds; the radioactivity of TMP being the most reduced, particularly low on the fifth and eighth days after implantation (Gerstein *et al.*, 1978). The above alterations may be caused by a hindered thymidine phosphorylation and/or the stimulation of its catabolism. Indeed, the activity of thymidine kinase from spleen of 22a hepatoma-bearing mice was found to be sharply reduced—from 1.32 ± 0.37 to 0.074 ± 0.03 nmoles of TMP formed/mg protein.

Suppression of DNA synthesis in the spleen of mice with 22a hepatoma correlates with a two- to threefold reduction of the organ's size. In the thymus and liver of mice with 22a hepatoma, unlike spleen of the same

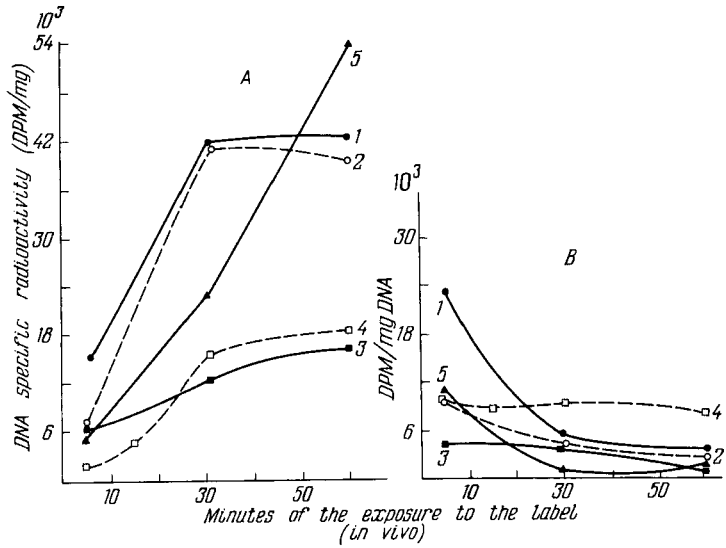

FIG. 8. Incorporation of ^{14}C-thymidine into the spleen DNA (A) and acid-soluble fraction (B) of tumor-bearing mice: 1, control; 2, on the second day after implantation of 22a hepatoma; 3, on the fifth day; 4, on the eighth day; 5, 48-hepatoma-bearing mice.

TABLE II
DISTRIBUTION OF THE RADIOACTIVITY IN THE ACID-SOLUBLE FRACTION IN PERCENT

Tissue	Term after tumor implantation	^{14}C-thymidine label		
		Thymidine + products of its catabolism	TMP	TTP
22a hepatoma	5 days	30.3 ± 3.2	3.8 ± 3.3	65.9 ± 5.7
	8 days	44.0 ± 3.4	6.6 ± 0.9	49.4 ± 2.8
48 hepatoma	2 months	75.0 ± 2.7	20.0 ± 2.6	5.0 ± 0.4
Liver	Control	69.0 ± 1.5	19.0 ± 1.3	12.0 ± 0.7
Spleen	Control	64.0 ± 5.5	25.0 ± 3.5	11.0 ± 2.0
Spleen of 22a hepatoma's host	2 days	71.0 ± 5.8	15.5 ± 3.9	13.5 ± 3.2
	5 days	81.5 ± 4.5	5.5 ± 2.5	13.0 ± 2.0
	8 days	80.0 ± 3.5	5.3 ± 2.4	14.7 ± 3.4
Spleen of 48 hepatoma's host	2 months	60.0 ± 10.0	27.0 ± 7.3	13.0 ± 3.2

animal, no changes in the incorporation of thymidine into DNA and distribution of the label in the acid-soluble fraction occurred. Other authors, however, reported a significant stimulation of DNA synthesis in spleen of mice and rats with tumors of various loci (Morgan and Cameron, 1973). One can infer that reactions of the host to the growing tumor are multiform and nonstereotypic depending on the type of neoplasm, its growth rate, and the animal species.

C. Lipid Metabolism

As we have demonstrated above, the functioning of a malignant neoplasm as a glucose trap leaves in certain measure an imprint also on the state of other types of metabolism, nitrogen metabolism in particular. We shall now consider the alterations in lipid metabolism that occur in the host in the process of tumor growth. Hyperlipidemia and the depletion of tissue lipids have long been noted in animals with tumors (Haven and Bloor 1956); moreover, hyperlipidemia begins to show particularly clearly at the moment the mass of tumor (Walker, 256) equals 10% of the host weight, diminishing, however, during the terminal period, when the mass of the neoplasm is doubled. At the same time Costa (1973) noted that Krebs mouse carcinoma-2 induces a 50% diminution of the lipid content of the host tissues as early as 7 days after transplantation, still not having grown large.

There is a statistically reliable (607 meq/liter versus 358 meq/liter) or

an almost twofold increase in concentration of unsaturated free fatty acids in the plasma of 40 patients with malignant neoplasms of various loci (Mays, 1971). However, the author does not consider this phenomenon specific for cancer because he observed still higher hyperlipemia in noncancer patients, who suffered from other chronic diseases that led to a catastrophic loss of their weight.

Lipidemia, owing to the elevated level of trigliceride-rich, very low density lipoproteins (CVLDL) in tumor-bearing animals and cancer patients, was reported (for references see Kralovic *et al.*, 1977) to be a result of the depletion of fat deposits. The above authors demonstrated that growth of rat Walker-256 carcinoma is accompanied by mobilization of free fatty acids, which precedes or parallels the depletion of carcass neutral lipid at the early stages of tumor development when its mass is yet small. Kralovic *et al.* (1977) noted an increase in the size of the adrenal gland of the host at this time, suggesting some stress. From our point of view these phenomena may be a result of hypersecretion of glucocorticoids enhancing gluconeogenesis in the host.

We shall now dwell on studies (Lankin, 1971, 1973 a,b; Lankin and Neyfakh, 1973; Polyakov *et al.*, 1977; Lankin *et al.*, 1976, 1977) in which the changes in the lipid content of host tissues were investigated in four models of mouse tumors, viz., ascites form of Ehrlich mouse carcinoma, sarcoma 37, sarcoma 180, and Walker carcinoma 256.

A rapid depletion of the fat deposits was observed, with a steady decrease of total lipids in the omentum and skeletal muscles up to the terminal period when, in Ehrlich carcinoma, it fell to 11% and 40% of its initial value, respectively. No changes were recorded in the brain and kidney lipids, while in the liver and blood plasma the increases proved to be extreme, reaching their maximum on the fifth day mainly through enrichment of the liver with triglycerides, and of blood plasma on the seventh day after transplantation. The liver of animals carrying various tumors displayed highly elevated levels of fatty acids as constituent of total lipids (Table III), by day 7 or 9 particularly these alterations were extreme. An enormous increase in the relative content of higher unsaturated fatty acids, oleic and linoleic, could be observed in the blood plasma during the period of intensive tumor growth, i.e., between the fifth and eighth days, and a progressive diminution of these acids in the omentum.

The depletion of the fat deposits and increase in the transport form of lipids (triglycerides) in the plasma and liver, as well as in free unsaturated fatty acids, attest to intensive mobilization of lipids caused by the tumor growing in the host.

The tumor apparently utilizes for its needs mainly free fatty acids

TABLE III
CONTENT OF FATTY ACIDS IN TUMOR HOST LIPIDS[a]

Liver of	Fatty acids in percent		
	16:0	18:1	18:2
Control animals	100	100	100
Mice with Ehrlich ascites carcinoma (the seventh day)	141	322	138
Mice with 37 ascites sarcoma (the seventh day)	163	424	162
Mice with Crocker ascites sarcoma (the seventh day)	209	233	133
Rats with Walker 256 carcinoma (the ninth day)	167	294	187

[a] Lankin (1973a).

(Spector, 1967) supplied by the host in complex with serum albumin or as a constituent of VLDL. At the same time the tumor itself does not exhibit any appreciable accumulation of lipids; nor is there any ketonemia in the host. This suggested to the researchers that the tumor itself used the mobilized lipids as the main source of energy, oxidizing them to CO_2 and H_2O (Mays, 1971; Lankin, 1973a,b; Spector, 1975). We, however, consider such interpretation unlikely. The ability of the tumor to oxidize lipids was in its time demonstrated in experiments *in vitro* with an excess of oxygen in the medium. As a matter of fact, *in vivo* the tumor is unable vigorously to oxidize large amounts of lipids because it is under conditions of progressing hypoxia (Vaupel, 1974; Tannok, 1976; Shapot, 1976). The main source of energy for it is anaerobic glycolysis (Shapot, 1975).

There are reasons to assume that the mobilization of lipids, hyperlipidemia, is a result of hypoglycemic "pressure" exerted by the tumor on the host. The mobilized lipids are assimilated by the tumor (as precursors of its membrane lipids), but mainly by the host tissues which, owing to a deficiency of glucose intercepted by the tumor, switch over in large measure to oxidation of fatty acids; especially since hyperlipidemia is of itself capable of limiting utilization of glucose by muscular tissue in conformity with Randle cycle but inducing at the same time the synthesis of enzymes involved in gluconeogenesis (Weber *et al.*, 1966). Hyperproduction of glucocorticoids, which is characteristic of the tumor-affected organism, acts in the same direction, i.e., favoring utilization of fatty acids (FA) as a source of energy and reducing assimilation of glucose by muscle tissue. The concentration of unsaturated FA in the blood,

mobilized from the deposited fat and other tissues, depends on the level of glucose in the blood. Hyperglycemia induced by administration of glucose into the organism completely blocks the mobilization of nonesterified unsaturated FA (Gordon *et al.*, 1957; Dole, 1956, see also Waterhouse *et al.*, 1969). A recent study on mice with Ehrlich ascites carcinoma (Baker *et al.*, 1978) confirms the above observations. Tumor-bearing animals had elevated FA fasting levels and on being refed glucose-rich food displayed inhibition of FA mobilization.

Even in the case where the fasting plasma-free FA concentration in patients with cancer was normal, induction of high hyperglycemia was required to suppress plasma FA levels (Edmondson, 1966).

On the other hand, any influence that hampers metabolism of glucose, for example, administration of 2-deoxyglucose (Laszlo *et al.*, 1960), favors mobilization of FA.

Thus hyperlipidemia and depletion of the fat deposits observed in the tumor-affected organism, although nonspecific of cancer, since these phenomena may be induced by other factors, including starvation, are conditioned by the parasitic character of tumor growth.

Mobilization of lipids and elevated amounts of higher polyunsaturated fatty acids circulated in the tumor host may entail an increase in the risk of blood coagulation (Gjesdal, 1976).

Prostacyclin (PGI_2) is known to prevent platelet aggregation. Accumulation of lipid peroxydes, e.g., 15-hydroperoxide of arachidonic acid, a potent inhibitor of PGI_2-synthesizing system (Moncada *et al.*, 1976) of endothelium and other tissues (Dembinska-Kiec *et al.*, 1977), would favor blood coagulation through enhancement of the formation of thromboxane (TXA_2) since both PGI_2 and TXA_2 derive from the common precursor—cyclic peroxide of PGG_2 (*Prostglandins*, 1977). TXA_2, synthesized in platelets, lung, and stomach, causes aggregation of platelets, vasoconstriction (Zmuda *et al.*, 1977), and eventually thrombosis.

Thus, impairment of lipid metabolism in the host may provoke imbalance in the blood coagulative–anticoagulative system.

III. Effect of the Tumor on Biological Characteristics of the Host Tissue

A. Enzymes

Since Greenstein's classic experiments (1947) with spontaneous and transplantable mouse and rat tumors, it has been known that the activity of catalase in the host liver is suppressed and that it becomes normal

again after surgical removal of the tumor. Later it was revealed that the tumor inhibits the synthesis of this enzyme and not its activity.

According to Kushiwagi *et al.* (1972), the liver of the mouse with ascites hepatoma AH-49 H exhibits a sharp decrease in incorporation of labeled leucine into the polypeptide catalase chains that form on polyribosomes precipitated by antibodies against the pure enzyme preparation.

At one time Shapot *et al.* (1963) found, besides water-soluble catalase, another form of this enzyme firmly associated with the lipoprotein of liver cells. It is extracted by *n*-butanol after preliminary removal of the soluble form and accounts for more than two-thirds of the total activity of the liver catalase. In the experiments with highly malignant Guelshtein ascites hepatoma 22a transplanted to C3HA mice we showed that the activity of only the water-soluble catalase diminishes in the host liver, while that of the lipid-bound enzyme does not change. The ratio of the second form of catalase to the first therefore increased from 2.5 to 4 (Davidova *et al.*, 1970).

Interesting phenomena pertaining to the changes in the activity of 23 liver enzymes in animals with slowly and rapidly growing Morris hepatomas, as well as Walker 256 carcinoma, were noted by Herzfeld and Greengard (1972). The growth of Walker carcinoma in 10 to 14-day-old sucklings prevented the appearance in their livers of the enzymes corresponding to the given stage of differentiation, for example, ornithine aminotransferase, glucokinase, glutamine synthetase, and malate-NADP-dehydrogenase. At the same time, premature induction of ornithine aminotransferase by glucocorticoids was observed in the liver of these animals.

After transplantation of tumors to adult animals, the content of those enzymes increases in their livers, the activity of which is relatively high in rapidly growing hepatomas and in embryonic liver. Some of these enzymes (tryptophan oxygenase, tyrosine aminotransferase), as the authors point out, are particularly intensively induced by glucocorticoids, and the increase in their activity must therefore be regarded as rather a nonspecific phenomenon associated with stress.

Those enzymes that are barely active or are entirely absent in hepatomas and embryonal liver such as ornithine aminotransferase and glutamine synthetase diminish. Hexokinase isozyme III proved to be an exception to this rule. It is very low in embryonic liver and is very active in hepatoma. It is precisely because of the increase in its content in the host liver that the total hexokinase becomes more active.

Our findings (Shapot *et al.*, 1976) agree with those of Herzfeld and Greengard (1972) and Farron (1972). In the livers of mice with a rapidly growing Guelshtein hepatoma 22a, the total activity of hexokinase and

its isozyme III sharply increased and the activity of glucokinase, the marker enzyme of the liver, diminished 66%–75%.

We took notice of one more feature. In tumors, as was pointed out above (Section II,A), glucose is maintained at an extraordinarily low level. In our endeavor to come as close as possible to the actual conditions existing *in vivo*, we discovered that in the medium containing 0.1 m*M* glucose, i.e., 2% of physiologic, the activities of hepatoma 22a hexokinase I and II are low, and only one isozyme III, characterized, unlike the other two, by a particularly high affinity for glucose, is the most active.

In the liver of mice with hepatoma 22a, we observed the same tendency, viz., at a low concentration of glucose in the medium the highest activity was that of isozyme hexokinase III (Fig. 9), the activity of all three hexokinase isozymes at a physiologic glucose concentration significantly exceeding that of the normal liver.

According to Teras and Isok (1974), the activity of total hexokinase in the liver of mice with Guelshtein hepatoma 22a increased 2.5-fold on the 28th day of tumor growth and that of glucose-6-phosphate dehydrogenase 2-fold, whereas the activity of glucose-6-phosphatase diminished 50%.

In the liver of rats with Morris hepatomas the activity of lysosomal enzymes—β-galactosidase, arylsulfatase, β-glucuronidase, cathepsin, and acid ribonuclease—increases (Shamberger *et al.*, 1971). These findings agree with those of Schersten *et al.* (1971) concerning the increase in the activity of the same lysosomal enzymes in the liver of 12 patients

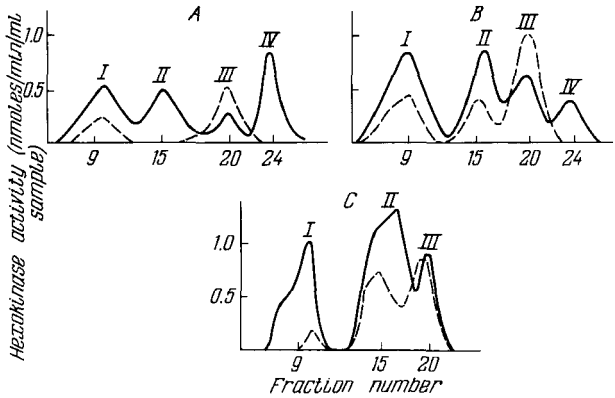

FIG. 9. Chromatography of mouse liver hexokinase isozymes on the DEAE-cellulose column on KCl concentration gradient. (A) Normal liver, (B) liver of mice with Guelshtein 22a hepatoma, (C) 22a hepatoma cells. Solid line shows enzyme activity in 5.5 mmoles/l glucose; broken line shows enzyme activity in 0.1 mmoles/l glucose.

with kidney cancer (see also Lundholm *et al.*, 1976). Jaroszewicz *et al.* (1976) examined 13 aminotransferases of the liver of rats with Guerin carcinoma. Of these the activity of valine, isoleucine, and methionine aminotransferases were increased 50%.

Earlier we (Krechetova *et al.*, 1972) found that latent endoribonuclease of membrane-bound liver ribosomes belongs to their structural proteins being localized in the small subunit. These ribosomes labeled *in vivo* with RNA ^{14}C-orotic acid, after 48 hours of incubation under conditions optimal for RNase, split their own RNA 80%–85%. On the other hand, endogenous ^{14}C uridine-labeled RNA of membrane-bound ribosomes of Zajdela hepatoma, rat hepatoma 27, and mouse hepatoma 22a under the same incubation conditions remained intact, which indicated the absence of endogenous ribonuclease activity. As a matter of fact, the enzyme could not be isolated from membrane-bound ribosomes of the above tumors, the possibility of inactivation of RNase by an inhibitor being excluded. Endogenous RNA breakdown in membrane-bound liver ribosomes of hepatoma-bearing rats on incubation was barely detectable.

A partially purified preparation of ribonuclease from membrane-bound ribosomes of the liver of tumor carriers proved to be far less active than that from the corresponding ribosomes of normal liver (Table IV), whereas the activity of the enzyme preparation from free ribosomes of the tumor host did not change.

Further purification of RNase from membrane-bound ribosomes of normal liver by chromatography on a DEAE-sephadex-A-50 column showed that the main activity was eluted with 0.15–0.17 *M* NaCl (Fig. 9) the specific activity of the enzyme increasing 200- to 250-fold. In this fraction obtained from Zajdela hepatoma membrane-bound ribosomes, no activity was detected at all, whereas in the case of membrane-bound ribosomes from the liver of animals with Zajdela hepatoma the activity was very low (Fig. 10), making no more than 3% the normal value (note the difference between the DPM scales in Figs. 10A and B).

At the same time we observed no essential difference in the activity of RNase of free ribosomes eluted from the column with NaCl 0.17–0.22 *M* solution (main activity peak) among the normal liver, the liver of a tumor carrier, and hepatoma. It follows that the activity of RNase of membrane-bound ribosomes from the liver of the tumor's host changes in the same direction as in the hepatomas themselves (Sukhova *et al.*, 1978), although these changes are not so strongly pronounced.

In contrast to that, RNase activity of membrane-bound ribosomes from rat regenerating liver (48 hours after partial hepatectomy) turned out to be even 25% higher than that of control.

A malignant human tumor very appreciably affects the activity of

TABLE IV
ACTIVITY OF ENZYME PREPARATIONS FROM RIBOSOMES IN ARBITARY UNITS (EU)

Source of enzyme	Membrane-bound		Free	
	EU	Percentage as compared with the liver	EU	Percentage as compared with the liver
Rats:				
Liver (normal)	25–33	100%	12–18	100%
Animals with Zajdela hepatoma	1.4–4.9	9.3	8.8–14	76
Animals with 27 solid hepatoma	6.0–8.0	24	14–18	100
Ascites Zajdela hepatoma	0.0–0.6	1	8.6–12	70
27 hepatoma	0.0–1.5	2	12	80
Mice:				
Liver (normal)	16.0–18.0	100	19.1–21	100
Animals with 22 hepatoma	6.0–8.5	25	10–14	70
Animals with 48 solid hepatoma	5.3	18	15.5	77
Ascites 22a hepatoma	0.0–0.54	1	11.6–17.0	71
Solid 48 hepatoma	1.1–3.0	7	12–16	70

pyrimidine nucleoside kinases of the nuclear sap of liver cells uneffected by metastases (Borzenko *et al.*, 1977). Normally the activity of these enzymes in the liver is negligible, but in the tissue of hepatocellular cancer the activity of thymidine kinase increases 40- to 50-fold and that of uridine kinase 120- to 150-fold. The activity of these enzymes in the liver of patients with gastrointestinal cancer changes in the same direction, but less strongly (Table V).

Herzfeld and Greengard's findings (1977) fully agree with the above data concerning thymidine kinase of the liver of cancer patients. The increase in thymidine kinase activity in the liver of rats with transplanted Morris hepatoma 7777 and lymphoma RVC 290 was enormous, in the first case exceeding normal activity 5-fold and in the second case 100-fold. It is interesting that with the growth of other transplantable tumors, for example adenomasarcoma CCC5 and Walker carcinoma, the activity of thymidine kinase in the host liver increased only negligibly.

We were surprised to discover, specifically for lymphoid tissue of rats, a sharp decrease in adenosine deaminase activity after Zajdela hepatoma implantation (Gerstein *et al.*, 1978); in the liver of these animals the

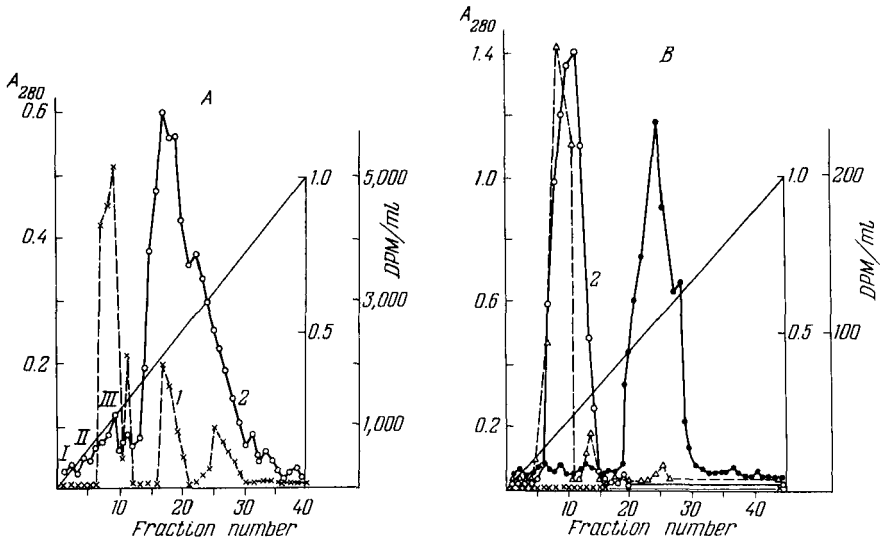

FIG. 10. Fractionation of the RNase preparation from rat liver and hepatoma membrane-bound ribosomes on the DEAE-column. (A) Enzyme preparation from normal liver ribosomes: (×--×) enzyme activity, (○——○) A_{280}. (B) Enzyme preparation from the liver of hepatoma-bearing rats: (△---△) enzyme activity, (○——○) A_{280}; from Zajdela hepatoma cells: (×××) enzyme activity (zero), (●——●) A_{280}. Enzyme activity was measured as DPM of the degraded ^{14}C-labeled ribosomal RNA preparation used as substrate.

activity of adenosine deaminase hardly changes (Fig. 11). Diminished deamination of deoxyadenosine may lead in the lymphoid organs, spleen and thymus, to selective accumulation and subsequent phosphorylation of the latter to deoxyadenosine triphosphate (Carson *et al.*, 1977), which is capable of suppressing DNA synthesis through the inhibition of ribonucleotide reductases (Reichard, 1968) and TMP kinase (Vornovitskaya *et al.*, 1968, 1972).

It is natural to assume that such impairment of metabolism in lymphoid organs must affect their normal functioning as a part of the immune defense system of the host.

Lawson *et al.* (1977) studied the synthesis of urea and the activity of carbamylphosphate synthetase in Morris hepatomas of various rates of growth and differentiation, as well as in the liver of tumor carriers. The activity of carbamylphosphate synthetase proved to be sharply diminished in the liver of rats with large, slowly growing hepatomas characterized by intensive synthesis of urea. Experiments with parabionts (healthy rats and tumor-carrying rats with a low activity of the liver enzyme) yielded no indication as to the presence in the blood of the latter of any factors affecting its activity.

Here we shall emphasize that not all the tumors examined by Lawson *et al.* (1977) suppress the activity of host liver carbamylphosphate synthetase. It was clearly marked in the case of heptomas 5123D, 21, and 47C in which urea formation is retarded, whereas in the liver of rats with hepatomas 20 and 9618A the activity of the enzyme was close to normal. It follows that in these studies we encounter again a phenomenon of *nonstereotypic* effect of malignant tumors on enzyme characteristics of the host tissues.

It is, furthermore, noteworthy that tumor carriers in certain instances exhibit diminished inducibility by xenobiotics, zoxazolamine and pentobarbital, of microsomal enzymes that catalyze the primary reactions of their metabolism; this observation undoubtedly reflects a tendency toward reduction of rough endoplasmic reticulum, i.e., the membrane-bound ribosomes we noted previously with respect to the liver of rats

TABLE V
NUCLEOSIDE KINASES IN THE LIVER NUCLEAR SAP: ^{14}C-TMP AND ^{14}C-UMP FORMED[a]

Source of tissue	Thymidine kinase	Uridine kinase
Healthy persons (n = 10)	4,192 ± 992.1	1,654 ± 723.4
Hepatomas	150,400	240,800
	212,950	374,500
	187,312	287,300
Liver metastases	142,217	192,405
Cancer of stomach		
Stage II	5,638	889
	4,874	1,002
Stage III	12,997	15,850
	8,070	9,874
	10,000	13,211
	8,950	13,200
	7,480	11,100
Stage IV	39,714	14,840
	63,801	48,849
	45,759	54,875
	59,714	68,211
Cancer of small intestine		
Stage IV	21,570	10,651
Cancer of cecum		
Stage II	18,244	15,424
Cancer of duodenum		
Stage I	1,975	3,489
Stage IV	75,424	79,500

[a] In cpm/mg protein.

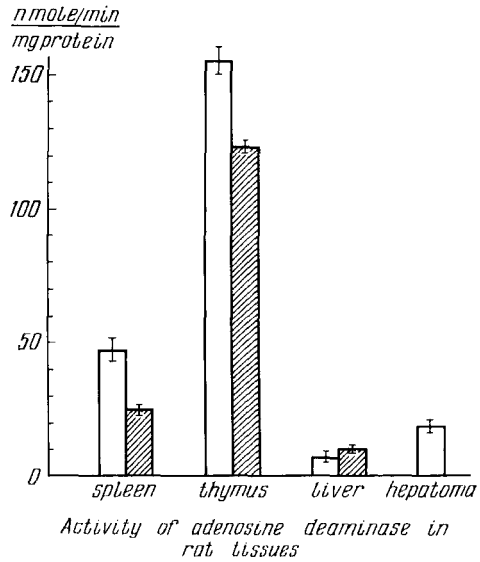

FIG. 11. Adenosine deaminase activity in tissues of normal and Zajdela hepatoma-bearing rats. Activity of the enzyme was measured in the cytoplasmic fraction, obtained by centrifugation of the nuclei-free tissue homogenate in a buffer containing 0.05 *M* tris-HCl (pH 8), 0.25 *M* sucrose, and 0.15 *M* KCl, spectrophotometrically by determination of the initial velocity of the reduction in absorption at 265 n*M* in the Unicam AR 25 Linear Recorder. Reaction proceeded at 37°C in the 3 ml solution containing 0.1 *N* sodium phosphate (pH 7.0), 4×10^{-5} *M* adenosine, 10 ml protein fraction (30 to 60 μg protein). The 0.019 difference in the absorption value was assumed to correspond to 1 μg adenosine deaminated. (□) Control, (▨) tumor-bearing rats.

with Zajdela hepatoma (Section II,B). Diminished inducibility of microsome enzymes was discovered by Rosso *et al.* (1971) in their studies of animals with ten models of rapidly and slowly growing tumors, viz., Walker carcinoma, solid and ascitic forms of Flexner-Jobling carcinoma, sarcoma 45, Guerin carcinoma of uterus, Yoshida rhabdomyosarcoma AN-130 and 602, sarcoma 180, Ehrlich solid and ascites carcinoma.

The microsomes of tumor carriers' liver metabolized zoxazolamine, aniline, amidopyrine, and *p*-nitroanisol much more poorly than did the microsomes of control animals. This warranted the conclusion that impaired in the tumor host are the reactions of aryl- and alkyl hydroxylation, as well as *N*- and *O*-demethylation. As a result, animals with the above tumors, especially females, turned out to be much more sensitive to the paralyzing action of zoxazolamine and the hypnotic action of pentobarbital. For example, after administration of a standard dose of pentobar-

bital, females with sarcoma 180 slept ten times longer than did healthy animals. After surgical removal of the tumor the metabolism of xenobiotics in the host liver was normalized.

As a result of the imbalance between the systems of generation of lipid peroxides (NADPH-dependent microsomal dioxygenase), on the one hand, and its inhibition ($\bar{O}_2$-dismutase) and the utilization of the above peroxides (GSH-peroxidase, GSSG-reductase), on the other hand, the microsomes and mitochondria of the liver of animals with Ehrlich ascites carcinoma and primary sarcoma induced by 3,4-benzpyrene accumulate excessive amounts of lipid peroxides (Lankin *et al.*, 1977).

The excess of lipid peroxides produces a toxic effect on the microsomes, i.e., the activity of the inducible enzyme, 3,4-benzpyrenehydroxylase is sharply suppressed and the level of cytochrome P-450 diminishes. In the mitochondria the activity of monoaminoxidases decreases, but the ability to deaminate adenosine monophosphate, and coenzymes containing this nucleotide, NAD, CoA, etc., increases (Khuzhamberdyiev *et al.*, 1973).

It is probably precisely the accumulation of peroxides of unsaturated fatty acids that accounts for the partial uncoupling of respiration and phosphorylation in the mitochondria of the tumor carriers' liver (Shapot *et al.*, 1976; Morozkina and Shapot, 1976).

The effect of the slowly growing sarcoma 45 and rapidly growing Walker carcinoma on the energy metabolism of mitochondria of tumor carrier's liver, isolated under maximum sparing conditions, and then incubated with succinate or glutamate, was studied by the polarographic method. The extent of dissociation of respiration and phosphorylation increased with the stage of tumor growth and in accordance with the degree of its malignancy. A decrease in the ADP/O coefficient and the respiratory control—diminished stimulation of oxygen consumption after addition of ADP—was observed. The NAD-dependent link of the respiratory chain proved to be the most vulnerable. The uncoupling of respiration and phosphorylation could to a certain extent be normalized if the mitochondria of the tumor carrier's liver were incubated with serum albumin which, as is well known, easily binds lipid peroxides and unsaturated fatty acids.

The above interpretation seems to be supported by the data of Chan and Higgins (1978). The *in vitro* aging of rat liver mitochondria was accompanied by an increase in the fatty acids levels with a concomitant uncoupling of oxidative phosphorylation. Replacement of the medium by 0.25 *M* sucrose containing 1% defatted bovine serum albumin reduced the free fatty acids levels and restored oxidative phosphorylation (raised

respiratory control index and ADP:O ratio). A substantial fraction of ^{125}I-iodinated serum albumin was shown to bind to mitochondrial membranes under these conditions.

Last, we shall mention a characteristic shift in the spectrum of lactic dehydrogenase (LDH) isozymes in all the tumor-unaffected tissues of cancer patients we studied (Shapot, 1975) jointly with Gorozhanskaya. These organs clearly show a diminished share of isozyme I, a change in the direction of the cathode fractions, and an increase in the coefficient (Gorozhanskaya and Shapot, 1971) which reflects the quantitative ratio of isozyme V to isozyme I activities (Table VI). We noted an enormous increase in the coefficient V:I for all the malignant tissues, whereas in benign neoplasms this coefficient does not differ from normal (Gorozhanskaya and Shapot, 1971).

Similar results were obtained by American authors (Hawrylewiez *et al.*, 1974) concerning the influence of malignant mammary tumor on portions of tissue of the same organ not affected by the neoplasm. Recalculation of these authors' data for the purpose of deriving the aforesaid coefficient showed that normal tissue of the mammary gland is charac-

TABLE VI
TISSUE LDH—I AND V ISOENZYMES IN PERCENT TO THE TOTAL LDH ACTIVITY

Tissue	*n*	I	V	V:I
Kidney				
Control	15	43.2 ± 1.6	2.9 ± 0.6	0.06
Cancer patient	16	34.7 ± 1.9	5.5 ± 0.9	0.16
Carcinoma	17	14.4 ± 2.0	22.7 ± 2.9	1.57
Stomach				
Control	15	30.1 ± 1.9	7.0 ± 0.5	0.23
Cancer patient	40	22.8 ± 1.4	10.2 ± 0.7	0.44
Carcinoma	45	3.7 ± 0.4	23.1 ± 1.0	6.20
Lung				
Control	15	20.5 ± 1.0	12.3 ± 1.7	0.60
Cancer patient	28	9.8 ± 1.1	12.8 ± 1.0	1.30
Carcinoma	25	6.3 ± 1.3	26.7 ± 1.8	4.30
Mammary gland				
Control	6	14.1 ± 1.0	9.3 ± 0.5	0.66
Cancer patient	30	5.7 ± 0.9	5.7 ± 4.6	1.00
Carcinoma	30	3.7 ± 0.6	32.1 ± 4.6	9.10
Uterus				
Control	6	11.0 ± 2.0	7.0 ± 0.6	0.63
Cancer patient	30	9.0 ± 1.2	7.8 ± 1.0	0.79
Carcinoma	30	5.4 ± 0.6	18.2 ± 1.7	3.70

terized by a coefficient that is close to 0, malignant tissue by a coefficient of 5.2, and the portion of the gland distant from the tumor has a coefficient of 0.36. In the latter case appear cathode isozymes IV and V which are usually absent in the normal gland, while isozyme I diminishes about 66%.

The above phenomena denote a tendency toward dysdifferentiation of specialized tissues manifested in changes in enzyme characteristics inherent in normal specialized tissue.

B. Cytoplasmic Informational RNAs and RNP Complexes

There are reasons to believe that abnormalities in many biological characteristics of distant host tissues induced by the growing tumor may be to a certain degree conditioned by certain features in the multistep process of gene expression. Such deviations are obvious in transplantable hepatomas but could be revealed in the liver of tumor-bearing animals as well, although they are less pronounced.

No appreciable differences in DNA-like RNA (D-RNA) between normal rat liver, Zajdela hepatoma, and liver of hepatoma-bearing rats could be detected in fractionating them by ultracentrifugation on a sucrose gradient and by chromatography on a methylated albumin Kiselgur column (MAK) using a salt and temperature gradient which permits separation of RNAs according to their molecular weight and nucleotide composition (Ellem and Sheridan, 1964; Lichtenstein and Shapot, 1971). Corresponding sedimentograms and chromatograms proved nearly identical.

However, drastic differences were revealed on studying cytoplasmic nonribosomal RNAs of the three tissues examined. To selectively label both nuclear and cytoplasmic D-RNAs, a partial actinomycin D block (low doses) was used arresting the synthesis of ribosomal RNAs but not affecting D-RNA synthesis. It turned out that liver cytoplasmic D-RNAs were far more heterogeneous in hepatoma liver and in tumor-bearing animals than in normal rat liver.

On MAK chromatography all the cytoplasmic D-RNA of normal liver, unlike nuclear D-RNA of the same tissue (eluted at an elevated temperature), was released in the salt fraction, i.e., was represented by a population of molecules loosely bound to the adsorbent and of relatively low molecular weight.

In contrast to that in the two other tissues a significant proportion of cytoplasmic D-RNA was eluted both in the salt and "temperature" fraction, the latter representing RNA tightly bound to the adsorbent (Shapot

et al., 1977). A similar heterogeneity in cytoplasmic D-RNA of hepatoma and liver of tumor-bearing rats was revealed by sedimentation characteristics (Lichtenstein *et al.*, 1978). The above findings indicate a reduced selectivity in the nucleus to cytoplasm transport of D-RNA in these tissues as compared with normal liver.

What kind of factors may be involved in producing such an effect? Schumm and Webb (1975) perfected the model system operating *in vitro*, which permits study of alterations in the transport of mRNA from nucleus to cytoplasm depending on the nature of the cytosol which serves as incubation medium. These authors using competitive DNA:RNA hybridization techniques demonstrated that the mRNA transferred from normal liver nuclei to the homologous cytosol differs significantly from that transferred from the same nuclei to the hepatoma cytosol.

Particularly impressive are the findings of Schumm and Webb concerning a sixfold stimulation of the transport of mRNAs from normal rat liver nuclei and broadening of their spectrum in the presence of a partially purified protein fraction obtained from the blood plasma of tumor carriers. Twenty-five percent of the mRNA sequences transported in the medium containing the plasma protein fraction from normal animals turned out to be different from that transported in the presence of the corresponding fraction from the plasma of tumor-bearing animals. These experiments carried out on animals bearing Morris 9618A and 5123D hepatomas and Novikoff hepatoma yielded identical results.

Let us mention one more observation (Shapot *et al.*, 1977) concerning the transport of D-RNA from nucleus to cytoplasm. The dynamics of the cytoplasmic D-RNA specific radioactivity under a complete actinomycin D block of nuclear RNA synthesis ("chase") was studied. In normal liver this block leads to a rapid decline in the specific radioactivity of cytoplasmic D-RNA, whereas in liver of tumor-bearing animals it remains unchanged in hepatoma, being even somewhat elevated. Under the same conditions the radioactivity of nuclear RNAs dropped in all three tissues studied.

The same regularity was established for poly(A)-containing cytoplasmic D-RNAs. A complete arrest of RNA synthesis induced a rapid reduction of their content in normal liver but not in the liver of hepatoma-bearing rats and in the hepatoma itself (Lichtenstein *et al.*, 1978).

The most reasonable explanation for the phenomena described would be that nuclei of hepatoma and of liver of tumor-bearing animals contain a large pool of cytoplasmic mRNA precursors which is able to maintain within at least 30 minutes the nucleus-to-cytoplasm transport at a constant level, unlike normal liver in which a nuclear pool of mRNA precursors becomes exhausted very rapidly once transcription is blocked.

Indeed, kinetic curves of the specific radioactivity of cytoplasmic D-RNAs under a partial actinomycin D block demonstrate that in hepatoma and liver of tumor-bearing animals these RNAs appear from the nucleus very soon and within 60 minutes reach the plateau, while in normal liver D-RNA transfer from the nucleus proceeds slowly, its content in the cytoplasm increases gradually and does not reach plateau even within 120 minutes (Shapot *et al.*, 1977).

There is one additional feature which is characteristic of hepatoma and liver of tumor-bearing animals. In hepatoma the proportion of rapidly labeled informosomes (postribosomal ribonucleoprotein complexes) was found significantly elevated at the expense of the reduction of polyribosomal mRNPs. This tendency is less pronounced in the liver of tumor-bearing animals. The above phenomena with selectively labeled D-RNA were revealed using three independent methods: by fractionation on sucrose concentration gradient, isopicnic ultracentrifugation in CsCl gradient, and finally by the technique used in our laboratory (Lichtenstein *et al.*, 1975), namely, nucleoprotein-celite chromatography permitting the separation of nucleoproteins on the basis of the tightness of the RNA-protein bonds. The latter method allowed us to obtain polyribosomal mRNPs, a very firmly built complex, and informosomes characterized by loose RNA-protein bonds in the opposite poles of the chromotogram. This feature, predominant proportion of informosomes in the tumor and liver of tumor-bearing animals, seems to be specific for them and is not found in regenerating liver. In addition, a shift in the polysomes/monosomes ratio toward the latter was observed in hepatomas and liver of tumor bearing animals (Shapot *et al.*, 1977).

All the deviations listed turned out to be typical not only of Zajdela hepatoma and the liver of its host but were verified in this laboratory on mouse Guelshtein hepatomas (Zborovskaya *et al.*, 1978).

C. Disorders of Endocrine Regulation

We have already mentioned the absence of gluconeogenesis stimulation in mice with Ehrlich carcinoma despite the profound hypoglycemia caused by the tumor. Special experiments have shown (Blinov and Shapot, 1974a; Blinov *et al.* 1975) that transplantation of this tumor sharply elevates the threshold of liver tissue sensitivity to the action of glucocorticoids which stimulate glycogenesis, gluconeogenesis and glycogen neogenesis. For example, whereas in healthy mice one day after administration of 5 mg of hydrocortisone the content of ^{14}C-glucose and ^{14}C-glycogen in the liver (radioactive alanine served as precursor) increased

6-fold and 115-fold, respectively, in tumor-bearing animals they increased only 3- and 2-fold. Moreover, under conditions that cause hyperproduction of glucocorticoids and other hormones that stimulate gluconeogenesis (various forms of stress) in the liver of these mice, gluconeogenesis remained at the initial level or even diminished 50% (paradoxical reaction), whereas in healthy animals the rate of gluconeogenesis doubled.

The elevation of the sensitivity threshold of gluconeogenesis to hydrocortisone was noted in the liver of rats with Zajdela hepatoma as well (Blinov *et al.* 1975). This phenomenon is very likely the result of a decrease in the content and the change in the properties of glucocorticoid cytosol receptors. As a matter of fact, K_{ass} to dexamethasone of the liver of rats 5 days after transplantation of Zajdela hepatoma diminishes from $(10^8 M^{-1}) \times 3.8 \pm 0.23$ to $(10^8 M^{-1}) \times 1.74 \pm 0.29$ ($p < 0.001$), while the number of binding sites decreases from $4.8 \pm 0.15 \times 10^{-13}$ mole/mg protein to $3.76 \pm 0.5 \times 10^{-13}$ mole/mg protein $p < 0.05$ (Dmitriyeva *et al.*, 1976).

It is curious that in some cases it is possible to reveal diminished reactivity of the tissues of the tumor host not only to hormones but also to other regulatory factors. It is well known that stimulation of gluconeogenesis in the liver may be induced by an excess of glucogenic amino acids of corresponding enzymes (substrate induction).

Control mice and animals with Ehrlich ascites carcinoma were injected with considerable amounts (0.6 gm/kg) of serine, as an inductor and with small quantities of ^{14}C-alanine, as a radioactive glucose precursor. In this case the rate of gluconeogenesis increased sharply, about threefold, in the liver of healthy mice, whereas in the liver of animals with the tumor gluconeogenesis it not only failed to be stimulated, but was even slightly depressed (Shapot, 1975a).

We also studied the changes in reactivity of cancer patients' lymphocytes to mitogens and hormones. On blast-transformation of peripheral lymphocytes induced by mitogens (phytohemagglutinin), three forms of RNA-polymerase are known to be stimulated. RNA synthesis catalyzed by A and B RNA-polymerases was studied under these conditions in isolated lymphocyte nuclei. The activity of these two RNA-polymerases in melanoma patients ($n = 18$) was higher than in donors ($n = 32$) 6 and 2 times, respectively; in lung cancer patients ($n = 18$) 5 and 4 times as high; and in sarcoma patients ($n = 20$) 7 and 8 times as high as in donors.

Unlike the RNA-polymerases of the donors' lymphocytes the activity of these enzymes in cancer patients was stimulated by phytohemmagglutinin to a lesser degree, on the average of 50% for form A and 65% for form B.

Dexamethasone inhibited the stimulated lymphocyte RNA-polymerase

A in donors and cancer patients about equally, while the sensitivity of RNA-polymerase B to the hormone was lowered 50%. It was also shown that the reduced sensitivity of the RNA-synthesizing system of lymphocytes to glucocorticoids develops in parallel with the dissemination of the melanoma, and it was proposed that the lowered reactivity of cancer patients' lymphocytes to glucocorticoids is conditioned by a decrease in representation of the T_1-cell subpopulation and its replacement by non-sensitive T_2-cells (Ioannesyants *et al.*, 1977, 1978).

It is probable that resistance of certain human leukemias and lymphomas to glucocorticoid treatment is due to the T_2-cell or B-cell origin of these neoplasms.

Further we (with Shelepov and Davidova) demonstrated functional disturbances in the insular apparatus in rats with two forms of hepatomas (see Shapot, 1978). Figure 12 shows that in normal rats (1.5 months old) and in rats with Zajdela hepatoma the maximum rise in the glycemic curve occurs in response to a glucose load, as was to be expected, on the 30th minute. In healthy animals a synchronous increase in immunoreactive insulin (IRI) in the blood was observed, its maximum also being reached toward the 30th minute. As for rats with Zajdela hepatoma, they

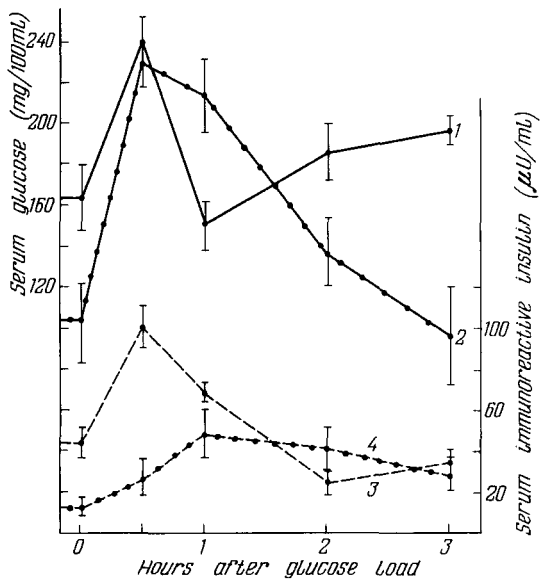

FIG. 12. Effect of the glucose load on the serum sugar and immunoreactive insulin (IRI) levels in rats. Glycemic curves: 1, control; 2, rats carrying Zajdela hepatoma. IRI curves: 3, control; 4, rats with Zajdela hepatoma.

are characterized by a relatively low initial IRI level in the blood serum and its delayed increase after a glucose load with also a delayed decrease, the initial IRI level failing to be reached even with 3 hours of observation.

The amplitude of reactive hyperglycemia at its maximum (in the 30th minute) in rats with Zajdela hepatoma turned out to be higher than in normal animals, but the assimilation of glucose by peripheral tissues was retarded.

An analogous study conducted on rats with solid hepatoma 27 found no initial hypoglycemia in these animals despite the elevated level of IRI. The assimilation of glucose after the load was also prolonged in these animals, and there was no synchronism in the peak of the glycemic curve and the IRI level; but, unlike the rats with Zajdela hepatoma, the deviations from the norm were, in this case, not stereotypic (Fig. 13).

From the data obtained, the mean rates of glucose assimilation were calculated (glucose tolerance test). In healthy rats this rate was 3.1 mg % glucose/min; in rats with hepatoma 27, 0.56 mg/100ml/min; and in rats with Zajdela hepatoma, 0.9 mg/100 ml/min. In the latter case the return of the glucose level from the reactive maximum to the initial one was prolonged to 138 minutes.

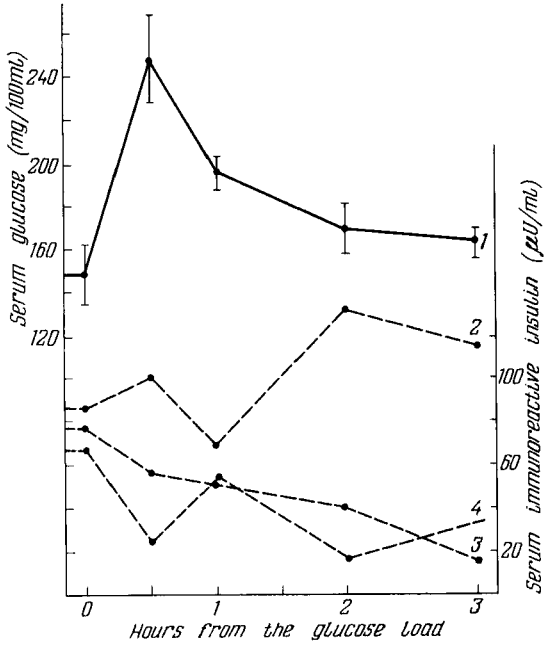

FIG. 13. Effect of the glucose load on the serum sugar and IRI levels in rats. Rats carrying solid hepatoma 27. Glycemic curve: 1: IRI curves: 2, 3, 4; different animals.

TABLE VII
SERUM INSULIN/GLUCOSE INDEX IN RATS AFTER THE GLUCOSE LOAD

Time after the administration of glucose (minutes)	Control rats	Rats carrying	
		Zajdela hepatoma	27 hepatoma
0	0.280	0.135	0.510
30	0.420	0.117	0.220
60	0.450	0.224	0.245
120	0.134	0.290	0.220
180	0.170	0.300	0.098

Table VII shows the changes in the insulin-glucose index (IRI in μU/ml to glucose in mg/100 ml) in tumor-bearing animals compared with healthy ones in the glucose tolerance test. The increase in the index in rats with Zajdela hepatoma, beginning from the 60th minute after the load, indicates that to assimilate an excess of glucose they require more insulin than normal; in other words, the sensitivity threshold of their tissues is elevated. In rats with hepatoma 27 attention is called to the extraordinarily high initial index.

Interesting observations were made by Teras (1979) on the changes in the inducibility of hexokinase, glucose-6-phosphate hydrogenase, and glucose-6-phosphatase by triiodothyronine (T_3) in the liver of mice with Guelshtein hepatoma 22a. On the seventh day of tumor growth the hormone caused a much greater increase in the activity of the above enzymes—8.5-, 3-, and 2.7-fold, respectively—than their induction in normal liver (supersensitivity!). However, a different picture was observed on the 28th day after transplantation of the tumor, viz., the liver nearly lost its sensitivity to T_3. The above phenomena obviously reflect a profound distortion of the reactivity of the host target tissue to T_3.

According to Horvath *et al.* (1975) induction of tyrosine aminotransferase by cortisol in the liver of chickens with transplanted hepatoma MC 29 is diminished. In the thymus of healthy chickens the activity of thymidine kinase decreases 80% after two injections of cortisol or dexamethasone in the course of 48 hours, whereas thymidine kinase of the thymus of chickens with hepatoma did not react to the hormone at all (Naray *et al.*, 1977).

Patients with stomach cancer ($n = 75$) exhibited a progressive (with the spread of the tumor process), statistically reliable decrease in the level of immunoreactive insulin in the blood serum from 18.5 ± 4.2. μU/

ml in early stages (versus 23.3 ± 4.0 in the control group with ulcer and gastritis, $n = 28$) to 11.8 ± 1.6 μU/ml in the III–IV stages (Vereschagina *et al.*, 1977).

The same authors noted as the most characteristic disorder in these patients, impairment of the inverse correlation between the production of adenohypophyseal hormone and the peripheral hormones. The content of the somatotropic hormone in the blood is sharply increased to 1.45 ± 0.43 mg/ml in the late stages versus 0.30 ± 0.01 in the control group.

The coefficient of thyrotropic hormone (TTH) to triiodothyronine in the blood increases from 0.85 (control group) to 1.5 with a tendency toward a decrease in the level of T_3 and an increase in the ratio of the total thyroxin to T_3. The authors interpret these phenomena to result from an overstrain of thyroid function and its eventual exhaustion.

Similar phenomena were discovered by Afrikyan (1977) in patients with lung cancer ($n = 102$), stomach cancer ($n = 92$), mammary cancer ($n = 26$), acute leukemia, lymphosarcoma, and myeloma ($n = 56$). Regardless of the localization and histogenesis of the malignant tumor the patients exhibited before treatment, an increase in the level of the thyrotropic hormone and a decrease in the concentration of T_3 in the blood with a coefficient of $TTH/T_3 = 2$ and higher. There were no such deviations in cases of benign neoplasms. Low T_3 syndrome was recognized also by other authors (Dessaint *et al.*, 1978) as characteristic of cancer patients.

In cancer patients, thyroid hypofunction apparently causes hyperplasia by the mechanism of negative feedback, overstrain of the adenohypophysis, which leads to depletion of basophils and subsequent dystrophy and even atrophy of the gland (Monastyrskaya, 1963). The results of histochemical studies of the thyroid and adenohypophysis of rats with transplanted tumors corroborate these conclusions (Morozkina *et al.*, 1975).

The reduced responsiveness of target tissues to corresponding hormones is likely to be characteristic of the tumor's host, although this feature may manifest itself differently in each given instance.

In women with mammary cancer, unlike healthy women, the elevated levels of estrogens during the menstrual (ovarian) cycle did not suppress the production of follicle stimulating hormone (FSH). No reduction in the blood FSH after administration of sinestrol into patients with mammary cancer in menopause could be observed. However, on remission induced by respective therapy an excess of sinestrol did suppress FSH production in such patients and failed to do it on recurrence (Sharoukhova, 1974; see Fig. 14). According to Dilman (1974), in patients with cancer of endometrium, mammary gland, and colon, a glucose load did

not reduce the levels of growth hormone in the blood. A paradoxical reaction to glucose load—hyperproduction of growth hormone—in patients with cancer of the endometrium was reported (Benjamin, 1974). In patients (n = 37) with the same localization of tumor the release of growth hormone in response to hypoglycemia induced by insulin proved very poor (Madajewicz *et al.*, 1977).

Patients with mammary cancer display elevated levels of blood cortisol which could not be suppressed by the administration of dexamethasone. The same phenomena were observed in patients with other forms of cancer. The author (Saez, 1974) draws attention to the abnormally high threshold in the hypothalamo-hypophyseal mechanism regulating the secretion of the adrenal steroids characteristic of patients with various forms of cancer. The feedback regulation of ACTH production by adenohypophysis is preserved, but higher amounts of cortisol are needed for this mechanism to be put in effect, particularly in patients with advanced cancer.

All the observations described leave no doubt as to the profound disorders in endocrine regulation provoked by the malignant tumor in the host as one of the manifestations of its systemic effects.

D. Immunodepression

Immunodepression doubtless is characteristic of both tumor-bearing animals and cancer patients, the degree of immune deficiency increasing with the neoplasm growth and dissemination. Nonspecific immune defense (a particular vulnerability to infection) as well as antitumor immunity seem frequently to be reduced (Harris and Copeland, 1974).

There are some indications that this phenomenon is a reflection of impairment of certain links of immunogenesis. The proliferation and differentiation of T- and B-lymphocytes are hindered. The growing tumor affects the cooperation of T and B cells. In addition, the activation of T suppressors which weaken both humoral and cell-mediated immunity was demonstrated in mice with Ca-755 (Petrov and Khaitov, 1977). Analogous observations were made in other laboratories as well.

Lung macrophages of rats with Walker 256 carcinoma growing in their lungs were shown to exhibit a sharply reduced capacity for phagocytosis, particularly at the advanced stages of tumor development. Phagocytosis was suppressed to the highest degree when the macrophages were incubated in the presence of the host serum. One to 3 weeks after tumor implantation, glucose oxidation by the host lung macrophages was strongly inhibited. In other words, a serious impairment of energy me-

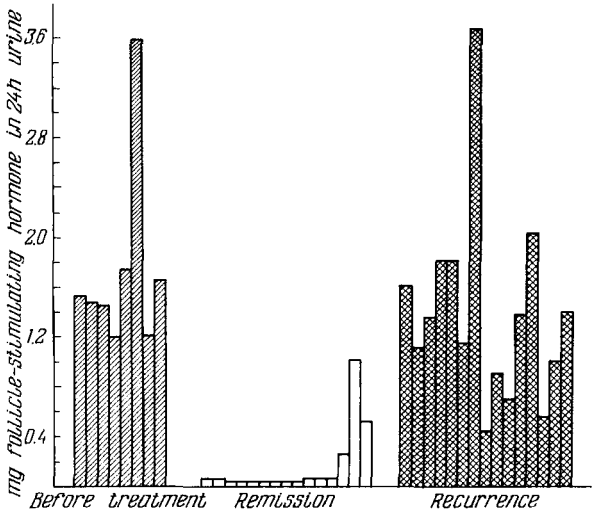

FIG. 14. Excretion of follicle-stimulating hormone by patients with mammary carcinoma after sinestrol treatment (Sharoukhova, 1974).

tabolism supporting macrophage functional activity was provoked by the tumor (Gudewicz and Saba, 1977).

It is of interest that very low density ($\rho = 1.006$) lipoproteins and low density ($1.006 < \rho < 1.063$) lipoproteins, isolated from cell-free ascites fluid of MM46 tumor, growing in syngeneic C3H/HE mice, suppressed lysis of this tumor by activated macrophages *in vitro* (Yamazaki *et al.*, 1977).

The exact reasons for the phenomenon of immune depression in the tumor host are not yet known; many factors seem to be involved. It is likely that certain substances are released in the circulatory system that interfere with immunogenesis. Normal human blood serum contains "immunoregulatory α-globulin" and related polypeptides capable of inhibiting various T-cell-mediated reactions: proliferations of lymphocytes in response to contact with specific antigens or mitogens as well as immunity of mice to syngeneic tumors, effector cells, or target cells being unaffected. "Immunoregulatory α-globulin" was found in particularly high concentrations in the serum of patients with metastasizing cancer and ascitic fluids (Wang *et al.*, 1977). Hence, an excessive synthesis of this α-globulin and related peptides, thus flooding the blood of cancer patients, may be one of the factors inducing immunodepression. The above authors believe that a reversible block of the process of "recognition" in the presence of "immunoregulatory substances" underlies the

loss of cytotoxicity to the syngeneic tumor by sensitized mouse lymphocytes.

The data on the immunosuppressive effect of the products of fibrinogen proteolytic degradation were reported by Girman *et al.* (1976). Dialyzable low molecular weight peptides (below 5,000 daltons) obtained by treatment of fibrinogen by thrombin were shown to suppress 98% blast-transformation of peripheral lymphocytes and sharply reduce the rosette-formation capacity of mouse lymphocytes immunized with sheep erythrocytes. Girman *et al.* examined 19 cancer patients and 3 patients with hepatocirrhosis. In most cases a correlation between the presence of fibrinogen degradation products in the serum and its immunodepressive activity was found, but in four patients no correlation could be revealed.

The serum of rats carrying Morris 5123D hepatoma exerted an immunodepressive effect inhibiting T-cell function first and subsequently that of B cells, this effect increasing with the enlargement of the tumor. The active factor, presumably an antigen-antibody complex, was thermolabile, nondialyzable, and could be removed from the serum by adsorption on lymphocytes (Schumm *et al.*, 1976).

With primary hepatoma and teratocarcinoma the part of an immunodepressive factor may be played by α-fetoprotein (Murgita and Tomassi, 1975a,b).

Let us turn to the possible effects of endocrine disorders and deviations in metabolism on the immune status of the tumor host. Hyperproduction of adrenal steroids in the tumor hosts occurs frequently as was mentioned above (Section II,B). According to Kendysh (1972), glucocoritcoids induce protein degradation not only in skeletal muscle but in the thymus and spleen as well, and thereby supply stimulated gluconeogenesis with amino acids as glucose precursors.

Involution of thymus manifested by a shrinkage of the gland and increasing destruction of thymus lymphocytes is a constant phenomenon in tumor-bearing animals, observed during the growth of Walker 256 carcinoma, Yoshida sarcoma, Zajdela hepatoma, benzpyrene-induced rat sarcoma, plasmacytoma of the Syrian hamster, spontaneous lung carcinoma, S-180 sarcoma, and mouse leukemia (Ertle, 1973). Before metastases occurred (Walker carcinoma), the involution of the thymus was reversible provided the tumor and adjacent lymph nodes were surgically removed (Toma and Simu, 1973). Thymus involution was regarded as a result of hyperfunction of the adrenal (Ertle, 1973) which may represent, from our point of view, a compensatory response of the host to the tendency toward hypoglycemia (see Sections II,A and B) induced by the tumor.

Kirikuta *et al.* (1970) point to the apparent analogy between the involution of thymus provoked by cancer and stress thymolytic atrophy, leading to an increase in the glucocorticoid levels in the blood. Bilateral adrenalectomy was shown to retard the involution of the thymus in rats carrying Walker 256 carcinoma.

A correlation between elevated 17-oxycorticosteroid concentrations in the blood and depressed immune reactions was found among patients with the most advanced mammary cancer (Saez, 1974).

The interception of nucleic acid pyrimidine nucleotide precursors, thymidine and uridine, from the thymus and spleen by the tumor (Section II,B) may have a direct bearing on immunodepression in the host. In addition, we recall our observation of a sharp decrease in the activity of spleen and thymus adenosine deaminase in rats with Zajdela hepatoma while its activity in their livers remains unchanged (Section III,A). Many hereditary combined forms of immune deficiency are known to be a result of the absence or a very low activity of the enzymes catalyzing the conversion of adenosine to inosinic acid, and particularly of the first one—adenosine deaminase (Giblett *et al.*, 1972). This defect leads to the accumulation of the deoxyadenosine (to be more exact, dATP) exerting a toxic effect on lymphocytes (Allison *et al.*, 1977; Carson *et al.*, 1977) and predominantly on T-cell population. It is quite possible that a reduced activity of adenosine deaminase has its share in the phenomenon, namely, causing T-cell dysfunction.

Indeed a reduced (50% to 25%) activity of adenosine deaminase in the blood of 321 cancer patients as compared with that of patients with benign tumors and other diseases was reported by Dinescu-Romalo *et al.* (1977). However, the above findings would have been more convincing were the measurements made directly on peripheral lymphocytes. Precisely this has been done by Uberti *et al.* (1976) on individuals with various types of solid malignant tumors. On the average the activity of adenosine deaminase in their lymphocytes was found to be substantially lower than that in lymphocytes of healthy subjects. Similar observations concering lymphocytes of patients with lung cancer were reported by Ogawa *et al.* (1977).

Finally, we will dwell on the data of Dudrick's group (Copeland *et al.*, 1977) as to the restoration of the impaired cell-mediated and humoral immunity in subjects with malnutrition by means of parenteral hyperalimentation, i.e., infusion of the solution containing glucose, amino acids, electrolytes and vitamins (A, D, E, C, B_1, B_2, B_6 niacin, and panthotenate). It may be expected *a priori* a benefic effect of the adequate parenteral nutrition on cancer patients who because of a parasytic character of tumor growth suffer from malnutrition besides impairment of intestinal

adsorption of enterally supplied food. Proceeding from the above reasons, Copeland *et al.* (1977) have studied rats with Morris 5123 hepatoma divided into three groups, kept on: (a) a carbohydrate-rich protein-free diet, (b) a standard diet, and (c) adequate parenteral nutrition. In animals of group (c) no stimulation of tumor growth occurred and, what was the most important, immunocompetency was fully restored in contrast to the other two groups.

Dudrick's group (Souchon *et al.*, 1975) showed in addition that adequate parenteral nutrition alleviated gastrointestinal toxicity of 5-F-uracil both in healthy rats and in cachexic cancer patients subjected to this chemotherapy.

The effect of adequate parenteral nutrition was then studied in emaciated patients with malignant neoplasms of various localizations. In 17 out of 23 cancer patients subjected to chemotherapy, who displayed impaired cell-mediated immunity, within 11 days of a systematically applied parenteral nutrition the skin test became positive and subsequent chemotherapy proved effective. Copeland *et al.* (1977) inferred that reduced immunity in the tumor host may be partly a result of malnutrition.

IV. Prospects for the Clinic

With due regard to the characteristics of carbohydrate metabolism of cancer cells in the host described above (Section II,A), it is possible to achieve a selective increase in their sensitivity to nonspecific antitumor agents, and thus be able to use them in smaller amounts and thereby relatively harmless to normal tissues by increasing the gap between toxic and therapeutic doses.

Numerous attempts made in recent decades to block the growth of tumors by suppressing glycolysis have, as is well known, failed. We (Shapot, 1968, 1970) already suggested the possibility of selectively sensitizing tumors to the action of damaging factors—chemical, radiation, and thermal—without inhibiting glycolysis, but by stimulating it to the utmost, creating through hyperglycemia conditions for the maximum saturation of the glycolytic enzymes. This idea also occurred to Ardenne and his associates (1972) who are still developing it in their studies. The gist of the approach is that by involving an enormous amount of glucose in metabolism of cancer cells (satisfying their high potential capacity for glycolysis that is not realized under usual conditions), the tumor accumulates an excess of lactic acid, which leads to sharp self-acidification of the tissue whose pH may diminish to 6.2 or lower, whereas the pH of normal tissues persists at the former level. In such a condition cancer

cells become particularly vulnerable to damage, probably because of an increase in the permeability of lysosomes membranes and the release from them of hydrolytic enzymes which are conducive to cell autolysis (Ardenne and Rieger, 1972). According to Ardenne's findings, chemical agents that affect membranes accelerate the above process and, consequently, intensify the antitumor effect of the complex therapy.

Hyperthermia also plays a role in complex tumor therapy, since many authors have shown cancer cells to be more sensitive to overheating than normal cells are. Grievous experience has shown that humans do not tolerate extreme hyperthermia, i.e., a temperature of 44°C, for which reason only moderate hyperthermia, not above 40°C, is used in treatment.

The attempts of the group headed by Aleksandrov and Fradkin at the Minsk Oncologic Institute (Savchenko *et al.*, 1977) to put into practice the above principles of selective sensitization of tumors in terminal and inoperable patients with far advanced and generalized forms of malignant tumors, melanomas, chondro- and osteosarcomas, and mammary cancer, in particular, seem to us very promising.

Maintenance of prolonged hyperglycemia for 6 to 22 hours at a level somewhat higher than 4 gm/liter, by administration of concentrated glucose solutions through a catheter into the superior vena cava proved to be optimum. Hyperglycemia is combined with (a) 4–5 hours of hyperthermia (41°C) with simultaneous cooling of the head and neck; (b) administration of various antitumor drugs; (c) administration of a thermosensitizer of cancer cells—naphtidon and vitamin A—as a lysosome sensitizer; and (d) irradiation. Endotracheal anesthesia with artificial ventilation of the lungs is necessary during the period of hyperthermia. The second "shock," i.e., chemotherapy or combined chemo- and radiotherapy in conjunction with hyperglycemia and brief hyperthermia, is administered 72 hours after the first session.

Today the procedures are so perfected that they are absolutely safe for the patient. It is possible to reduce the doses without diminishing the therapeutic effect; for example, those of antitumor drugs have been reduced 50%–92% and irradiation 90%–93%. Fifteen days after the procedures, and with local hyperthermia immediately afterward, the tumors diminish, necrosis occurs, and "shrinkage and drying-in" set in even when, under usual conditions, the tumor is resistant to chemo- or radiotherapy.

This treatment makes possible surgical removal of the remaining tumor nodes, which is necessary in order to prevent recurrences (Savchenko *et al.*, 1977).

Application of local hyperthermia (in cases of superficial tumors) by high frequency currents is particularly effective in the above complex of

treatment since it makes possible targeted heating of neoplasms to 42°–43°C.

By the end of 1976, combined treatment in its general and local variants had been administered to 80 patients with advanced forms of cancer considered "hopeless." The results can be judged at least by the following figures: 34 are still alive, 25 from 1 to 6 years. Thus it is obvious that the hyperglycemic background essentially enhances the effectiveness and selectivity of the usual therapy, especially when chemical, radiation, and thermal factors are combined.

It would be appropriate to devote some attention to one more clinically important aspect of the systemic action of tumors. A novel method of quantitatively evaluating the functional state of the liver by the kinetics of absorption and excretion of radioactive, labeled ^{131}I Rose Bengal with the use of cybernetic techniques was recently elaborated. This has made it possible to reduce the multiparameter diagnostic space, which formerly rendered difficult an unambiguous interpretation of results, to a unidimensional space (numerical classification or a physiologic scale of functions) (Sivoshinsky and Narkevich, 1975; Sivoshinsky *et al.*, 1977; Perevodchikova *et al.*, 1977). Many hundreds of patients with malignant neoplasms of nonhepatic localization exhibited, before treatment, an increasing depression of the functional state of the liver in the III and IV stages of the tumor development.

Surgical removal of the tumor or clinically effective antitumor treatment clearly improved the function of the liver. Thus evaluation of the extent of the tumor's systemic effect on the liver makes it possible to judge the results of the use of therapeutic factors and to test quantitatively the toxic side effects of the drugs. Of course, the above approach, which makes it possible to study only one of the many functions performed by the liver, cannot give an exhaustive appraisal; but as a test method, it has demonstrated its value in practice.

V. Conclusion

Here we should like to express some general considerations and assumptions which follow from the materials set forth in this article.

It is reasonable to assume that interception of glucose by the malignant tumor from host tissues which creates "hypoglycemic pressure" on the host is a phenomenon triggering a cascade of various metabolic and endocrine disorders in the host. Among them are: (a) hyperfunction of adrenals followed by the stimulation of catabolism of proteins as well as gluconeogenesis from amino acids, the latter becoming less available for tissue protein synthesis; (b) functional impairment of the insulin produc-

ing apparatus; and (c) mobilization of lipids with possible blood hypercoagulability as one of the consequences (see Section II,C).

It is most likely that the main form of systemic action of a malignant tumor on the host is its ability to compete successfully with the host tissues for the vitally important metabolites and other factors. Such an advantage may be partly conditioned by the characteristics of the surface membranes of tumor cells which favor unusually rapid transport of these metabolites from the capillary network to the intracellular space.

The text contains information to the effect that the rate of growth of transplanted malignant neoplasms directly correlated with their ability to intercept from the host tissues, including proliferating tissues (thymus, spleen), the precursors of nucleic acids—thymidine and uridine. As a result, the level of the latter in these tissues during the very first minutes after injection of the label proves to be particularly low, much lower than normal, and their incorporation of the corresponding nucleic acids is sharply retarded. It may be assumed that similar phenomena also occur with the exogenous amino acids supplied with the food, as well as arising in the process of breakdown of tissue proteins.

The assumed characteristics of the surface membranes of tumor cells may be regarded as a most important condition that determines the very possibility of the parasitic growth of the neoplasm in the host, as well as exerting systemic action on it.

One more factor may favor the uptake of vital metabolites by the tumor. With regard to glucose, we may take for granted the role of the enormous concentration gradient established between arterial blood and the tissue of the tumor. It is quite possible that a similar process takes place with amino acids and the precursors of nucleic acids, since in the tumor, unlike in normal cells, the catabolism of proteins and nucleic acids is almost completely suppressed. It follows that recyclization of amino acids and nucleotides does not take place in cancer cells (Jewell and Hunter, 1971), and this must reduce the pool of these compounds.

Moreover, it is not unlikely that the tumor utilizes the host endocrine system to accelerate the transport of glucose, amino acids, and nucleosides from the blood. Receptors for insulin were found in the plasma membranes of Zajdela ascites hepatoma (Shelepov, *et al.*, 1978).

We consider successful competition of the tumor with the host tissues the main form of its systemic action because it is precisely this form that is, in the final analysis, able to cause not only essential changes in the carbohydrate, nitrogen, and lipid metabolism in the host and transform the protein-synthesizing apparatus of the protein-secreting organs, but also the tension of the compensatory mechanisms, including the functions

of the endocrine system, which often ends in its exhaustion and even the dystrophy of various endocrine glands.

Both these systemic effects of the tumor on the host (see Sections II and III) seem to be interwoven. Hypoglycemia developed in those tumor hosts that were not able to sufficiently stimulate gluconeogenesis because of the elevated threshold of the liver to controlling hormones.

As we also have seen, it is possible to draw a connection from the competitive abilities of the tumor and suppression by it of adenosine deaminase activity in the lymphoid organs to the phenomenon of immunodepression in the host.

The changes in the activity of some of the enzymes whose synthesis is regulated by hormones may be the result of changes in the hormonal balance; for example, an increased production of glucocorticoids which induce the synthesis of thymidine kinase in the liver and inhibit it in the thymus and spleen.

We should like to place special emphasis on the enormous prospects of using in oncological treatment adequate parenteral nutrition (Wretlind, 1972; Dudrick *et al.*, 1977; Sudjyan, 1973a,b, Sudjyan *et al.*, 1975, 1977) as a potent means of counteracting the impoverishment of the host tissues in vital trophic factors and the disturbance in its homeostasis, including immunodepression. It is now already clear that parenteral alimentation can serve as an essential adjuvant of surgery and chemotherapy, overcoming the toxic side effects of drugs. Regrettably, these possibilities are sometimes underestimated by oncologists.

A good deal in the systemic action of tumors is not clear as yet: for example, the elevation of the sensitivity threshold of the target tissues to corresponding hormones and other factors that control their functions. This latter phenomenon involves a weakening or complete disruption of the negative feedback between the central and peripheral endocrine glands which normally safeguard their functional potential.

Many so-called complications of the main disease in cancer patients require special analysis. Here pathophysiologists, immunologists, biochemists, and clinicians still have a vast and as yet unexplored field of endeavor. We shall venture to dwell briefly on two of these complications. It is possible that the frequent paradoxical combination of hemorrhages and thromboses in cancer patients is a result of deposition of fibrin in the vascular bed of such tissues as the liver, spleen, and heart valves with subsequent fibrinopenia (McKay *et al.*, 1953; Khato *et al.*, 1977; Goodnight, 1974).

Tendency to bleeding may be associated with an increased consumption of platelets and a reduced half-life of fibrinogen (Rasche and Dietrich,

1977), their turnover rate being four times as high as normal (Schlichter and Harker, 1974). However, at the same time the hypercoagulable state of the blood is a phenomenon most concomitant to cancer.

Most patients examined with squamous cell and poorly differentiated lung cancer (Sopotzinskaya, 1978) exhibited elevated levels of blood fibrinogen. The above author found a direct correlation between the elevated concentrations of blood fibrinogen and free cortisol.

In addition, excessive amounts of thromboxane following inhibition of the formation of prostacycline as a result of hyperlipidemia may be causatively involved in the phenomenon of hypercoagulability of the blood of cancer patients (see Section II,C).

As for dermatomyositis and polymyositis, there are reasons to suspect their autoimmune nature (Curth, 1974). Cytotoxic action of the lymphocytes of cancer patients with polymyositis with respect to muscle explants in culture, as well as fluorescent antibodies in the bulk of muscle fibers of these patients, has been found (Frion, 1974). Such phenomena might be rendered comprehensible by some experimental observations. Organ-specific antigens of renal and muscular tissues have been found in the extracts of several rat hepatomas, and antigens of muscular tissue have been discovered in extracts of renal adenocarcinoma (Fel and Schwemberger, 1968). Johnson *et al.* (1965) found striation, and the presence of myosin, revealed immunologically, in the Wilms tumor—a neoplasm of renal origin.

It is conceivable that malignant human neoplasms of various histogeneses are able to synthesize and eject ectopic muscle-type proteins through the plasma membrane into the blood, thereby inducing formation of corresponding antibodies, which damage the patient's muscles, the foregoing being conditioned by an anomalously functioning immune system. It follows that the emergence of an autoimmune disease in cancer patients is one more indication of impairment of the regulatory mechanisms of their immunologic supervision.

An analysis of the multiformity of manifestations of the tumor's systemic effects makes it possible to contemplate the solution of the old controversy of whether a tumor should be considered a local or general disease of the organism. A benign tumor has no systemic action. If it does not, by its bulk and localization, hamper the functions of vitally important organs, as is the case, for example, with benign brain tumors, the life span of the host does not diminish. There is therefore every reason to regard benign neoplasms as a purely *local* disease.

On the other hand, the development of even a nonmetastasizing malignant tumor impairs the activity of all physiologic systems of the host and disturbs its homeostasis. Hence it is obvious that here we have to deal

with a *general* disease of the organism, although it is induced and maintained by local neoplastic lesions.

Usually the degree of the tumor's malignancy is evaluated, especially by experimenters, by the rate of its growth. It seems to us that the following formulation is more exact: the degree of malignancy is higher the sooner the tumor causes the death of the host, i.e., the shorter its life span. Here we imply both the dissemination and systemic effects of the tumor.

We assume that the systemic effects of a tumor is one of the *obligatory* properties of malignant neoplasms, exactly as are invasiveness and destructive growth. Unlike this, cataplasia and metastases, although characteristic of malignant neoplasms, do not manifest themselves in all cases.

It is time we corrected the concept of the tendency toward resemblance of the biochemical characteristics of distant tissues of the host to those of the tumor itself. This rule is far from being always observed. However, if the directions of the changes in the biochemical characteristics of the tissues and tumor really coincide, this coincidence warrants our assumption that the above deviations are *not essential* to the tumor transformation in the sense that distant tissues do not in these cases acquire the basic property of a neoplasm, i.e., the capacity for uncontrolled growth.

Last, there is one more conclusion pertaining primarily to experimenters. Since practically no single organ of the tumor-carrying organism functions normally, it is necessary, in studying any characteristics of a malignant neoplasm, to use, as control, tissues only of a healthy animal, and in no case those of the host. Incidentally, clinicians would also do well to take this factor into consideration. Let us recall here the aforesaid changes in the spectrum of lactic dehydrogenase isozymes in the tissues of oncologic patients not directly affected by the tumor.

In conclusion, we should note the amazing feature of the systemic action of tumors, namely, its *nonstereotypic* character. It cannot be expected that the impairment of function of any one physiologic systems will be similar, even with the same type of tumor. The disorders may differ in the extreme and may not always be predictable.

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