ANALYSIS OF THE MECHANISM GOVERNING MITOGENETIC RADIATION OF THE LIVER IN MICE WITH IMPLANTED CANCERS

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In a previous work [6] we demonstrated stimulation of peptide synthesis in the liver of starved mice, using mitogenetic radiation. At that time we postulated that in the liver of normally fed healthy mice, where the cells are not sources of mitogenetic radiation [3] because of the absence of fluorescent substances in them [9], peptide synthesis is accomplished by the expenditure of great energy quanta. The latter are derived from the recombination of free radicals of atoms, occurring as rare phenomena during the enzymatic processes.

This hypothesis gave rise to another: if the liver of mice with implanted cancers contains cancer "extinguishers," which we would be led to believe on the basis of the data of S. Ya. Zalkind and M. B. Novikov [7], the formation of these great quanta of energy would be inhibited. Thus, peptide synthesis would probably be impeded and the state of the liver in these animals would be analogous, in this regard, to the state of the liver in the starved animals.

The following work was devoted to the experimental verification of this hypothesis.

METHOD

Inasmuch as the presence of cancer "extinguisher" in an aqueous extract of the liver [7] may be caused by its presence in the blood, it had to be accurately proven that the cancer "extinguisher" was actually present in the liver cells. The following method was developed for that purpose.

The freshly extirpated liver of a mouse with an implanted tumor was ground up with powdered quartz. The paste obtained was agitated in physiological saline three times, and centrifuged each time. One ml of the supernatant fluid was drawn off and three "transfers" were made to a glycocoll solution, following the method of A. G. Gurvich [5]. Every third "transfer" was tested for the presence of cancer "extinguisher." After the last centrifugation a small amount of the sediment (0.5 ml) was homogenized in a glass homogenizer, and 3 "transfers" to a glycocoll solution were also made from the homogenate. The 3rd "transfer" was again tested for the presence of cancer "extinguisher." Determination of the "extinguisher" was carried out according to the method of A. G. Gurvich [5]. We used biological detectors to observe the mitogenetic radiation.

RESULTS

Cancer "extinguisher" was detected in the 3 "transfers" after the 1st and 2nd centrifugation, not detected in the 3 "transfers" after the 3rd centrifugation, and again encountered in the 3 "transfers" of the homogenate. Microscopic study of the paste before the homogenizations showed that the powdered liver tissue consisted of such small groups of cells and their fragments that it was hardly possible to retain the extinguisher present in the blood after two washings. Thus, we concluded that the cancer "extinguisher" was actually contained in the liver cells.

TABLE 1

Determination of "Extinguisher" in the Blood and in the Liver of Mice following Subcutaneous Implantation of a Malignant Tumor Fragment or Subcutaneous Injection of Ascitic Fluid (exposure 6 sec; effects expressed in percent)

Nature of the test	Interval following implantation or injection				
	20 hr	20 hr	44 hr	68 hr	10-11 days
Radiation from the irradiated glycocoll solution	54	47	49	48	66
The same, with the addition of the 3rd "transfer" from the blood or supernatant fluid after the 2nd centrifugation	2	2	3	0	2
The same, with the addition of a boiled 3rd "transfer" from the blood or supernatant fluid after the 2nd centrifugation	52	41	41	55	54
The same, with the addition of the 3rd "transfer" from the blood or supernatant fluid after the 3rd centrifugation	–	58	55	28	52
The same, with the addition of the 3rd "transfer" from the homogenate		2	+2	-2	-2
The same, with the addition of a boiled 3rd "transfer" from the homogenate		42	43	46	66

In line with this, we developed a new hypothesis concerning a possible mechanism for the debilitation of peptide synthesis in the liver of the mice. Since innumerable enzymatic processes go on in the liver, accompanied by the production of these large energy quanta, the number of these quanta must also be relatively great, and thus, the cancer "extinguisher," whose concentration is extremely low, would not be able to completely prevent their arisal. Hence, in this case only a partial extinguishing must occur. It could be postulated that as a result of such a partial extinguishing an inadequate number of the large energy quanta arises, and thus, some of the polypeptide molecules do not undergo further consolidation. This results in the loss of the molecule's ability to fluoresce.* Hence, an acculumlation of fluorescent substance occurs in the liver. These substances are capable of taking up the unextinguished energy quanta and giving them off in the form of photons of mitogenetic radiation. In other words, it is quite probable that the liver of mice with implanted tumors possesses the power to radiate mitogenetically.

In order to experimentally verify this hypothesis it was of primary importance to determine the times at which the "extinguisher" appeared in the blood and in the liver, to investigate the liver of the animals with implanted tumors for radiation, and, where a positive test result was obtained, to determine the time of its appearance.

According to our observations, cancer "extinguisher" appears in the blood even within 20 hr after the subcutaneous implantation of a tumor fragment in the mice. These data coincide well with the results obtained back in 1938 by A. G. Gurvich and L. D. Gurvich [4]. According to those latter results the "extinguisher" appears in the blood on the 1st–2nd day after implantation of a tumor fragment. The cancer "extinguisher" can also be detected in the liver 20 hours after the tumor implantation; it remains in the liver for a prolonged period (investigation after 44, 68 hr and 10-11 days yielded positive results). The data obtained are presented in Table 1.

* It is known from experimental data that protein molecules do not act as fluorescent substances.

TABLE 2

Determination of "Extinguisher" in the Liver Cells of Normally Fed Healthy Mice 20 Hours After Injecting 1 ml of Natural Cancer "Extinguisher" (6 second Exposures)

Nature of the test	Effect (in %)
Radiation from the irradiated glycocoll solution	76
The same, with the addition of the 3rd "transfer" from the supernatant fluid after the 2nd centrifugation	8
The same, with the addition of a boiled 3rd "transfer" from the supernatant fluid after the 2nd centrifugation	61
The same, with the addition of the 3rd "transfer" from the supernatant fluid after the 3rd centrifugation	39
The same, with the addition of the 3rd "transfer" from the homogenate	3
The same, with the addition of a boiled 3rd "transfer" from the homogenate	58

The figures in the 1st and 2nd columns represent averages of three trials, in the 4th—averages of 2 trials. In the 3rd and 5th columns are shown data from single determinations.

The effect in percent (as in Table 2 as well) is computed according to the formula: [(E—C)/C]·100, where E—experimental, and C—control.

However, according to the data of A. G. Gurvich and L. D. Gurvich [5], natural cancer "extinguisher" penetrates poorly into cells. Thus, we especially determined the presence of "extinguisher" in the liver of a healthy test mouse 20 hr after subcutaneous injection of a solution of natural "extinguisher" (Table 2).

As can be seen from Table 2, the experiments showed that natural "extinguisher" penetrates into the liver cells of a healthy mouse, and may be detected in them 20 hr after the injection.

Subsequent testing of the liver of animals with implanted tumors showed that the liver in these animals produces mitogenetic radiation. The average effect of 15 trials was 37%. This radiation was noted 22 hr after the mice were injected subcutaneously with 0.2 ml of ascitic fluid from Ehrlich's adenocarcinoma (investigations were not performed at earlier intervals). The spectrum of this radiation consisted of 2 bands—2060-2070 A and 2260-2270 A—characteristic of NH_2 groups, and 2 bands—1980-2000 A and 2440-2450 A—representing OH groups. In the mice with well developed tumors we observed these spectral bands at exposures 3 times shorter (3 sec) than the exposures necessary to record these bands in the spectra from the selective radiation of the livers of mice starved for 4-5 hr (10 sec). This reduction in the exposure time is evidence of an increase in the concentration of NH_2 and OH groups characterized by these spectra. Thus, we concluded that in the liver of mice with well developed tumors, as a result of the reduction in peptide synthesis, there prevail dissociative processes leading to even greater subdivision of the protein substrate than that which takes place in the liver of mice starved for 4-5 hr.

This conclusion is supported by biochemical investigations [8], in which it was shown that the liver of healthy test rats contains fewer amino acids (27.5 μM/g) and fewer amino acids linked to peptides (29.9 μM/g) than the liver of rats with Walker's sarcoma (34.6 μM/g and 45.7 μM/g).

Actually, this difference is probably even greater, since the liver cells of the starved animals [6] and the animals with implanted tumors (as indicated above) produce mitogenetic radiation, and thus, reciprocally radiate each other. At the same time, it has been shown with the aid of chemical methods [1] that 30 min irradiation of the liver cells in healthy, normally fed animals, using mitogenetic rays, increases their permeability, and, thus, their output of amino acids and peptides into the blood.

The results presented show that cancer "extinguisher," according to the basic hypothesis, can fully cause radiation of the liver, beginning with the earliest intervals of its appearance. This concept was supported by the following experiments.

One ml of hydrolyzed cancer "extinguisher" was injected into normally fed, healthy mice, whose livers, as was already indicated, do not radiate. Since the "extinguisher" solution always contained glycocoll in various concentrations (2.5×10^{-4}, 2.5×10^{-5}, 2.5×10^{-6}), due to the method of its preparation,* a solution of glycocoll was injected into the control mice in concentrations of 2.5×10^{-3}, 2.5×10^{-4} and 2.5×10^{-5}. In both series the liver was tested for radiation 10 min after the injection. The appearance of radiation was recorded in the control series only at a concentration of glycocoll equal to 2.5×10^{-3}, while in the experimental series liver

* "Transfer" to a saturated glycocoll solution, with subsequent dilution by a factor of 10^4, 10^5 and 10^6.

radiation arose with hydrolyzed cancer "extinguisher" solution injections containing glycocoll in concentration of 2.5×10^{-5} and even 2.5×10^{-6}.

However, in considering the question of which mechanism causes the arisal of mitogenetic radiation in the liver of the mice with implanted cancers, it is necessary to consider that this radiation may be caused by the accumulation of endogenous cancerogenic substance in the liver of these animals. This substance was shown to radiate by A. G. Gurvich and L. D. Gurvich [5].

The presence of this substance in the blood was demonstrated by us only after 4 days following the subcutaneous implantation of a fragment of Ehrlich's adenocarcinoma in the mice. We were convinced of this by the fact that the spectrum of radiation obtained for the blood was identical with the spectrum for endogenous cancerogenic substance which was established by E. S. Billig [2]. Thus, this substance could only cause the arisal of radiation from the liver at the indicated time interval.

Therefore, our hypothesis that the appearance of cancer "extinguisher" in the liver cells is sufficient to cause its mitogenetic radiation appears to have been justified.

In conclusion we would like to point out that we have not excluded the possibility that mitogenetic radiation from the liver of animals with implanted cancers is of a complex character, and consists of 2 and, perhaps, even 3 components: the 1st is caused by the appearance of cancer "extinguisher," the 2nd – the appearance of endogenous carcinogenic substance after 4 days, and, finally, the 3rd – a result of degradation processes. Degradation radiation has been described by A. G. Gurvich [5].

Thus, the question of the mechanism by which this radiation arises still remains unresolved, and is subject to further analysis.

SUMMARY

The liver of mice with implanted cancer produces mitogenetic radiation; its spectral analysis shows that the protein molecules of the cell substrate become smaller in size. After implantation of a piece of tumor in mice, the mitogenetic radiation of the liver and cancer "extinguisher" was detected in its cells in 20-22 hr, whereas endogenous cancerogenic substance was detected only after 4 days.

The results obtained are regarded as a confirmation of the hypothesis formerly advanced by the author of the role played by cancer "extinguisher" in the mechanism of mitogenetic radiation of the liver in animals with implanted cancers. The complexity of the problem and the necessity of its further study are emphasized.

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