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Problems of early cancer diagnosis and therapy

by

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I. — *NITRILOSIDES, PARTICULARLY AMYGDALINE* IN CANCER PROPHYLAXIS AND THERAPY*

For some time now, cancer research workers, particularly if open minded, have found it interesting to investigate why certain animal species, human races and human groups show a much lower cancer incidence than normally observed, or were even completely free of this disease although they fitted within the limits of a statistical criterium of longevity. Thus individuals with hyperthyroidism or « infectious diathesis » (UNGER) are better protected against cancer than normal collectivities ; the same seems to apply to individuals who during all their life receive continuously alliin and allicin supplied by garlic, or who breathe air containing a minimum of 10 mg/m³ of carbon disulfide.

Sufficiently reliable medical reports indicate that the Hunza tribe in Karakorum and the eskimos are practically free of cancer as long as they remain in their natural environment. Wild animals are very seldom cancerous, especially the herbivores. Particularly resistant to cancer are sheep, a phenomenon which has repeatedly led to various investigations.

E.T. KREBS Sr. and E.T. KREBS Jr. have studied for several decades the cancer resistance of certain tribes and animal species, and they have come to the conclusion that this resistance is due to a single cause which can now be considered as sufficiently reliable. A concise summary of these studies follows :

The Hunza tribe and the eskimos, in distinction to all other people, absorb large quantities of nitrilosides ; the Hunza draw this substance from apricot stones, and the eskimos from certain arctic berries and from the partly digested stomach contents of killed animals. Sheep, but also other herbivores, absorb large quantities of nitrilosides in their foder ; grass contains up to 40 gm of nitrilosides per kilo dry weight when it grows on land with poor vegetation.

* This subject was presented independently at the Sessions of the Society for Pre- and Postoperative Tumor Therapy in Bad Sulzuflen, on October 29, 1969, first by DEAN BURK, Director of the Department for Cell Chemistry at the National Cancer Institute, Bethesda, Md. U.S., and then by Hans A. NIEPER, Silbersee Hospital, Hannover. For better understanding, both communications will be presented here together.

Phylogenetically, nitrilosides are probably very archaic components that develop in plants, and which under the action of suitable hydrolyzing enzymes, for instance betaglucosidase, dissociate into HCN, benzaldehyde and a sugar ; the dissociation of amygdaline out of apricot stones produces glucose. It has been demonstrated in sheep that the dissociation of nitrilosides takes place in the stomach of the animal. Almost all the liberated hydrocyanide is absorbed but it does not produce the usual toxic effect since it is detoxified in the organism by available sulfur and namely, essentially ubiquitously in the tissues by means of an enzyme called rhodanese. This forms thiocyanate in the tissues.

Now it is important to note that the absorption of nitrilosides and the above described splitting process are not only more or less empirically present in human or animal groups almost free of cancer, but that the active therapeutic application of this agent reveals clearly interesting carcinostatic properties. This supports also the KREBS's concept as a whole.

NIEPER indicates in his communication that in his opinion nitriloside metabolism is possibly an ancient pragmatic phylogenetic principle which serves to protect a higher structured organism from the aggression of atavistically autonomized cells — and this is cancer.

It has thus been demonstrated in rats with Walker carcinoma that the inhalation of HCN produces a highly significant therapeutic action. Moreover, it has been shown in long-term experiments that the nitrilosides have a carcinostatic effect (extensive documentation). Dean BURK reported that experiments conducted on cancer cell cultures reveal that not only HCN alone but also the combination of HCN with benzaldehyde is to be considered as the active principle of nitrilosides. At any rate, the activity of HCN and of benzaldehyde are potentiated in Dean BURK's testing material. Furthermore, he was also able to prove that the effect of amygdaline on cancer cells is potentiated manyfold by an increase in the temperature of the nutrient from 37° to 40.2° and more. This supports the reliability of the well known discovery made by LAMPERT, and above all that of v. ARDENNE, but on the other hand, it supports also the hypothesis that amygdaline is a true cancer therapy agent. In the discussion of the anticancer activity of nitriloside splitting products, NIEPER's communications during 1962-1963 concerning his work with KÖHLER, and SCHMID and SCHMID are really noteworthy insofar as they indicate that investigations of the constitution and activity of carcinostatic sulfur compounds seem to indicate that thiocyanates are most probably compounds with true carcinostatic activity. Allicine (the active principle of garlic) and LK 92 (dioxymethylthiuramdisulfide), (KÖHLER and NIEPER) belong also to this group of sulfur compounds whose carcinostatic action derives from a specific basic structure. As a consequence of its instability, LK 92 raises problems in clinical long-term therapy — and only this type of application is interesting in view of the nature of the activity of these substances — while allicine produces a very strong and disagreeable smell. It is not yet clear whether the thiocyanate formation from nitrioloside splitting in the vicinity of every cell yields also a carcinostatic agent. However, this seems to be most probable*.

NIEPER's findings, which showed that the intravenous application of amygdaline (3 mg of Laetril daily i.v.) can be potentiated by complementary injection of beta-glucosidase (60-100 mg of Nutritional Biochemicals), are also remarkable. This phenomenon was revealed in three patients with lymphadenitis carcinomatosa and lung metastases. Further investigations conducted by NIEPER in three patients with bleeding, inoperable gynocological tumors, two with stomach carcinoma and two with lymphadenitis carcinomatosa seem to indicate that oral administration of amygdaline in compressed tablets is more active than the intravenous injection. This is even more clearly revealed when the equipotency of doses is compared (ratio from about 1:15 to 1:30, oral:intravenous). The reason for the improvement obtained with oral administration is probably to be found in the better splitting of amygdaline in the stomach. One fact, which must be taken into consideration, is that in the treatment of cancer, the active principle of nitrilosides is to be used mainly in prophylaxis and early protective therapy. This

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* In the meantime clinical trial with amygdalin and S.-donors in cancer patients make most probable that the resulting thiocyanate is the major factor in the carcinostatic activity of nitrilosides. Solubility in lipids and a powerful "etheric" polar function of the sulfur in thiocyanates (Sexton) make them ideal subcellular carcinostatic compounds in the sense of the principle described by NIEPER, KÖHLER, and XALABARDER in 1962/63.

stems already from the very nature of the activity of this method which demands extremely long-term therapy. On the other hand, the complete atoxicity of this method of treatment, which is maybe nothing else but a rediscovered natural principle, permits the unlimited use of this substance. The success of this therapy would therefore depend on very modern precocious or prophylactic cancer diagnosis, the feasability of which would require a rather profound reorientation in the mind of the medical profession and of the public. But the reviewer believes that this problem, which will be summarized by NIEPER in the following communication, can be solved if, as in the case of nitrilosides, an atoxic therapeutical agent can be offered in good faith. The consensus of the participants is that the splitting of nitrilosides as a basis for cancer therapy, which was discovered by KREBS and KREBS, is an important step forward in the struggle against cancer once its method of use, as described above, will have been well understood.

A more detailed communication on this subject will be presented in a few months.

Persons interested in receiving documentation
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II. — *PROBLEMS OR EARLY CANCER DIAGNOSIS WITH PARTICULAR EMPHASIS ON ELEMENTARY MECHANISMS OF CANCERISATION AND FORMATION OF MALIGNOLIPIN**

The key to successful cancer therapy is an efficient, early and precise diagnosis. This is not only when surgery or X-ray therapy is concerned, but much more so for future medical cancer therapy. For cancer is above all an internal general disease. The nitriloside method of tumor therapy described earlier has already shown that modern early diagnosis must fulfill very extensive requirements if nitriloside activity is to offer positive results to the largest number possible of patients or potential tumor bearers.

What is still being called « early detection » of cancer does not, in the opinion of the author, deserve its name. The only exceptions are, at best, early detection of cervical carcinoma or the beginning of localized oral tumors. Even in the case of early skin cancer, there are most likely other unrevealed tumor cells hidden in the skin (see Congress on Local Tumor Therapy, Turin, October 1969, and J. HARTUNG). When a tumor reaches a stage of development detectable by X-ray or isotopes, as a rule, more than half the time from the beginning of the disease to the death of the patient has already elapsed.

Basically, we shall, therefore, have to adopt early indirect methods of diagnosis and will have to renounce, if otherwise not possible, from the direct detection of the tumor itself. The less detectable a tumor because of its very small size, the better should be the chances of success with continuous *long-term therapy,* for instance, with nitrilosides. In the opinion of the speaker, every positive result of an indirect cancer test should imply immediate application of long-term carcinostatic therapy for indefinite periods of time. To be sure, the presently available alkylating cytostatics and antimetabolites are not suitable for such treatment since they are themselves carcinogenic, and weaken also the immuno-protection of the organism against cancer. Therefore, such long-term therapy must be applied with the methods e. g. recommended by Dean BURK and H.A. NIEPER in the preceding communication. The speaker sees in long-term therapy a treatment which can last for several years or even decades.

In the opinion of the speaker, we shall have to get used to treating cancer according to indirect diagnostic criteria.

We would certainly rather turn to indirect detection measures to fight off enemy planes or rockets, and not rely upon their visibility to the naked eye.

Only a few indirect methods of cancer diagnosis will be discussed here, which the speaker believes hold promises for the future.

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* Presented at the Sessions of the Society for Pre- and Postoperative Tumor Therapy in Bad Salzuflen, on October 26, 1969, by Hans A. NIEPER, Silbersee Hospital, Hannover, FRG, and summarized here.

It is clear that the setting up and interpretation of an indirect cancer test, i.e., produced by laboratory techniques, requires an approved concept or a valid model based on our present knowledge of the mechanism of cancerisation and the nature of cancer. The speaker presents here his cancerisation model which developed into its final form essentially after a visit to KOSAKI in Japan in 1966 who discovered malignolipin.

The speaker added that so far, three observations have been made in cancerisation that can be considered as essential and, therefore, absolutely specific as far as cancer development is concerned, for they are not observed in healthy tissues :

1) The loss of specific organ antigens during cancerisation. Thus, for instance, the loss of the hepatic antigen caused by the development of a hepatoma which was first described by Ernst WEILER, 1964. Later, FRIEDRICH-FREKSAN expressed the opinion that specific organ antigens are identical to the specific organiser — or organisation mediator — of cell structure, and their destruction must therefore lead to both functional and structural « undifferentiation ».

2) A phospholipid that differs from any known phospholipid of the healthy organism develops in all cancerisation processes, and also in the course of lymphogranulomatosis. This phospholipid, which KOSAKI calls « malignolipin », is characterized by multiple amino groups and binds much more firmly with hematoporphyrine and coproporphyrine III than any other phospholipid. NIEPER supports the concept that structurally malignolipin is primarily nother else than the skeleton remaining after the destruction of the organizing antigen, which is a protein. This would mean that malignolipin is part of the cell plasma structure responsible for the genetic information of malignant metabolism, possibly on a phylogenetically extremely old basis. Various observations made in malignolipin research would support this hypothesis, in particular, a surprising quantitative feed-back phenomenon observed by KOSAKI. Moreover, a malignant structure and its metabolism is specifically sensitive to a particular fraction of cobra venom which, as known, is a mixture of various phospholipidases. It would appear that the specific anti-malignolipin activity of this fraction isolated by BRAGANCA, leads to the inhibition of the malignant process. Furthermore, more than 60 articles have appeared in world literature over the past years that indicate that hematoporphyrine and its metal chelates have a carcinostatic effect, and this, based on an independent mechanism that differs from that of the usual commercially available cytostatics.
Evidently, malignolipin is functionally inhibited by the reaction with hematoporphyrine. On the other hand, the tagging of malignolipin with hematoporphyrine can also be used for the diagnosis of malignant tissue since during surgery such a tagged tissue mass fluoresces under a fluorescence lamp (Dorothy GRAY).

3) The last essential observation on cancerisation was made by R. WYBURN-MASON who showed that an independent autonomous structure develops in cancer cells under thermotropic conditions — a phenomenon that was seen in every single case of a study on more than 1,000 tumors — which then, when the cell membrane is ruptured, escapes, from the cancer cell into the body fluid stream where it leads an independent life. This happens primarily when the cell environment or the media surrounding the tissue are warmer than the test sample itself. This phenomenon had already been discovered earlier and had been investigated by electron microscopy (NIEPER and XALABARDER, 1962), but WYBURN-MASON presented sufficiently reliable proof of the essential relationship with malignancy.

NIEPER considers that on the basis of his earlier experience, the development of this autonomous subcellular structure in cancer is a structural autonomisation of a cytoplasmatic structure which is, in fact, the result of the earlier mentioned destruction of the organiser antigen. In this respect, NIEPER, as earlier Reiner MÜLLER, believes that finally cytoplasmatic structures are phylogenetically older than the cell as a whole and that in the case of the loss of the organiser, they can revert to a pre-protozoal phylogenetic heredity, if such is their motivation. This concerns both structures that correspond to primitive mycetozoans and the

metabolism which, we know, since WARBURG, to be cancer metabolism and which is similar to that of primitive yeasts or fungi.

Very noteworthy is the phenomenon that KOSAKI had already revealed in his first communications on malignolipin (Science, 1956, and Mie Med. J., 1956-1962), i.e., that the subcellular structure which, as mentioned before, escapes from the cancer cell, is particularly rich in malignolipin. This observation supports NIEPER's theory. It would indicate that the subcellular structures have a potential malignancy of their own and may, for instance, be responsible for the development of the cancer disease. In principle, this had already been discovered earlier, and had incited NIEPER and KÖHLER after 1957 to set up an infracellular or subcellular cancer therapy (see first communication).

The cytoplasmatic process in cancerisation, as described by NIEPER in this paper, has lately received supplementary evidence. Various research groups have been able to show in different studies that a defect in the genetic system of nucleic acid in the cell nucleus is not an essential feature of malignancy. In this respect, the most interesting investigation was carried on kidney carcinoma in the Leopard Frog. Cell reproduction tests did not reveal any genetic defects in these tumor cell nuclei, at any rate none of the dimension of malignancy induction (MCKINNELL, DEGGINS and LABAT, Science, 3891). According to his investigation of nuclei in hemoblastoses LÄGERLÖF came to the same conclusion. NIEPER believes, therefore, that the phospholipids represent a phylogenetically older genetic principle than the nucleic acids. This is, however, no original concept (ANSELL, HAWTHORNE). NIEPER is trying, therefore, to discover the genetic defect of cancerisation in the phospholipid structures of the cytoplasm, and he sees in malignolipin its morphological correlate. In this respect, tumors and the cancer disease are characterized by two parallel operating mechanisms, namely, first the cancer cell as the smallest unit of the tumor, and then the subcellular malignant agent rich in malignolipin which can essentially be regarded as the producer of the general malignant disease.

This hypothesis would imply that in cancer therapy first the treatment of cellular mechanism would have to be taken care of, for instance, with surgery, X-ray therapy, and also conventional cytostatic drugs, overacidification plus hyperthermia according to v. ARDENNE's method, etc... Also hydrogen cyanide and benzaldehyde obtained from the desintegration of amygdaline are active against the cancer cell as a whole, as has been demonstrated by Dean BURK.

As far as the second mechanism is concerned, cancer treatment must also comprise infracellular or subcellular cancer therapy directed against the autonomous agent. Immunity drugs can be applied here (malignolipin has the properties of a specific antigen), hydroxyl radicals have an agent inhibiting effect which lasts for 6 weeks after X-ray treatment, and finally the sulfur containing compounds synthetized specially by NIEPER and KÖHLER for subcellular cancer therapy, as well as allicine belong to this group. Also Atabrine and Chloroquine. Thiocyanate, which is formed in the organism from nitriloside breakdown has fundamentally also the same properties (see first communication). The necessity for simultaneous anticellular and subcellular therapy, and the clinical superiority of such a parallel method of treatment is best demonstrated in chronic myeloid leucemia (NIEPER, to be presented soon).

It must be remembered that autonomous subcellular malignancy can develop contagion properties in the sense of an infection. In addition to CLARK, GLOVER and ENGLE, this has also been demonstrated lately by HAMAZAKI during serological studies, and recently by LIVINGSTON.

The question that then arises is to establish what causes the destruction of the organising antigen, for its loss is the first essential general condition of any cancerisation. At the present state of our knowledge, the following factors are to be taken into account :

1) The chronic autoaggression (autoimmune aggression) against the organ since this is often directed against the specific organ antigen (SERAFINI, NIEPER, CAJANO). Clinically, the chronic autoimmune aggression is probably the most important factor in cancerisation.

2) Viruses with cancerigenic properties (herpes simplex, adeno, SV 40, etc.).

3) Chemical exogen and endogen carcinogens.

4) Physical carcinogens such as Roentgen radiation, isotopes, UV light, etc.

5) Feed back defects of the organising antigens due to functional insufficiencies.

6) Physiological disappearance of the organizing antigen caused by age? (SEIFERT).

7) Dysontogenetic defective arrangement of the organiser (dysontogenetic tumors).

It has been definite proven that the combination of these factors in the sense of a « co-carcinogenesis » can produce stronger and more rapid cancerisation of the cell. As far as the combination of chemical carcinogens with viruses is concerned, this has been demonstrated by L. ZILBER.

NIEPER has produced evidence on the dramatic co-carcinogenetic mechanism of auto-immune diseases plus adenovirus. In this respect, it must be noted that the autonomous agent of potential malignancy may require a continuous viral informant.

This is just about the actual stage of our knowledge in this field, on the basis of which methods of early cancer diagnosis could be developed and evaluated.

It would naturally be very interesting to discover the autonomous malignant agent in the blood. In this respect, the darkfield methods conceived by BREHMER and SCHELLER have gained some recognition. But their deficiency is that they cannot prove coherently whether or not the observed structure contains malignolipin. KOSAKI has isolated and confirmed the existence of malignolipin in cancer patients with an extraction method. The reliability of this detection method, according to his statements — which are quite plausible — was practically absolute in more than 1,200 cases. But this method is so elaborate that it cannot be applied to routine diagnoses. Therefore, NIEPER is now trying to detect the existence of malignolipin in its structural binding agent with a Zeiss fluorescent microscope equiped with a special filter whereby he is not forced to used the complicated extraction method. So far, these tests have not led to anything really decisive, but they will certainly not remain unsuccessful. The difficulty resides in the necessity to liberate also sufficiently the malignolipin in the structure so that it can react with hematoporphyrine, the more so since the hematoporphyrine must afterwards be washed out of other kinds of unspecific combinations. In this respect, it must not be forgotten then that the autonomous agent can obviously form important layers of mucoproteins starting from malignolipin, which prevent the access to the malignolipin surface. But on the other hand, the fact that the combination hematoporphyrine-malignolipin produces a somewhat different emission spectrum, which was discovered by KOSAKI, is certainly valuable. This permits to use in the fluorescent microscope a discriminating filter technique.

On their part, the mentioned mucoproteins seem also to play an important role in early cancer diagnosis. An experimental hamster tumor has been discovered whose cells divide only partly. But when serum from cancer patients with a little female sex hormones is added to the culture medium, the dividing process becomes normal. The blood factor in cancer patients that produces this phenomenon is a glycoprotein which develops outside of the tumor. Ailene HERRANEN has made a report on this subject.

The autonomous subcellular malignant agent fixes itself preferentially on erythrocytes, multiplies then inside and can thereby destroy them. This has been known for several decades to many participants of the Congress. This malignant agent cannot only be dyed, but the damage caused to erythrocyte membranes could be used as a criterium of cancer disease. NIEPER has used various osmotic concentrations for this purpose, and has measured then with a Coulter Counter the volume and number of erythrocytes that remained large. This method is however, too complicated and is burdened with factors of uncertainty. It is interesting to note that in cancer, the erythrocytes absorb larger quantities of rubidium. This may perhaps permit to develop a nuclear-medical method of early cancer diagnosis, as VOGEL et al. had already presented at the Cancer Congress in Tokyo in 1966.

All the methods described here apply diagnostically only to the « cancer disease » phenomenon and not to the « tumor ». Critics very often do not take into consideration this differentiation or fail to understand it.

Another possibility in early diagnosis is to accept the principle — described by the speaker — of phylogenetic atavism in cancerisation *propter hoc* and *post hoc* as an original point in favor of the method. Thus, Philip GOLD found an antigen in the blood of patients with intestinal cancer which is also to be found in the intestine, pancreas and liver of normal fetuses, but which disappears with maturation. This atavistic fetal antigen reappears only in the case of cancerisation.

Possibly the following phenomenon, which was first described by NOVARRO, belongs to the range of phylogenetic-hereditary relationships of tumor cells. The author has used this phenomenon systematically in early cancer detection. He discovered that tumors, even when very small, produce minimal quantities of chorionic gonadotropin (CGH). This substance, as known, is produced in great quantities by chorion-epitheliomas. The initial basis of the author's investigations was the more than 30-year old BEARD's trophoblast theory on cancer which, fundamentally, cannot resist all criticism even if the principle of phylogenetic heredity is thereby proven. It is possible that single tumor cells produce CGH in the course of certain precise phases of their atavistic regression. NOVARRO has conceived a special extraction method to recover small traces of CGH which requires the use of about 500 ml of patient urine. The CGH thus recovered is then determined immunologically with pregnosticon (Organon). The reliability of this method indicated by NOVARRO is supported by two other investigators, but substantially disapproved by a third.

The use of a modern, indirect and early method of cancer detection, whatever it may be, has only then sense when adequate consequences are drawn from positive results : immediate initiation of long-term prophylactic treatment even if the tumor remains unknown or undetected. Nitrilosides seem to be particularly suitable for such long-term protective therapy which, in the opinion of the speaker, is the only existing possibility for the ultimate control of cancer.