BIO-MEDICAL RESEARCH
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1 2	61 62
#11 Investigation of the Blastomogenic	21
Action of Polyvinyl Chloride Resin	22
Used in the Food Industry --	23
Neoplastic, Translation, Russian, Plastics,	24

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1 2	61 62
Onkologiia Informatisionen Bulletin,	31
Prilozhenie, No. 3, pp. 24-28, 1970	32

Brief Summary

1 2 10	61 62
SUMMARY:	61
	62
	63
	64

#11 INVESTIGATION OF THE BLASTOMOGENIC ACTION OF POLYVINYL CHLORIDE RESIN USED IN THE FOOD INDUSTRY.

M. Radeva and S. Dinoeva

Translation of: "Prouchvane Blastomogennoto deystviye na Polivinikhloridpa plastmasa, izpoluzuvana v Khranitednata Promishlenost," Onkologiia Informatisionen Biuletin, Prilozhenie, No. 3, 1970, pp. 24-28.

#11. INVESTIGATION OF THE BLASTOMOGENIC ACTION OF POLYVINYL CHLORIDE RESIN USED IN THE FOOD INDUSTRY

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A number of papers have been published in recent years on the development of tumors in animals on subcutaneous implantation of polymer plastics. The occasion for research in this direction was provided by the malignant tumors obtained by P. Turner in subcutaneous introduction of bakelite discs. The blastomogenic activity of synthetic polymers was subsequently confirmed in experiments by Oppenheimer, Druckrey, Hueper, Benerjee, Bering, Laskin, Nothdurft, N., Kogan, Shabad, and others.

The essential nature of the blastomogenic action of plastics has not been clarified. Among the various hypotheses advanced regarding the mechanism of such action we shall deal with the hypothesis of the chemical mechanism of the blastomogenic action of polymers, since it provided us with the occasion for the present study. According to this hypothesis a blastomogenic effect is exerted by the active radicals separated from the implanted plastic or by the active chemical centers formed after the separation. It is assumed that these radicals, in penetrating the cells, cause depolymerization of the nucleic acids and modify the activity of certain enzymes. A blastomogenic effect is also exerted by the depolymerization products separated from the macromolecular component of the plastic. The hypothesis of a chemical mechanism of "polymer" blastomogenesis is favored by the experiments of Oppenheimer with subcutaneous implantation of labeled ^{14}C polystyrene, polyethylene, and polymethylmethacrylate, in which radioactive substances indicating depolymerization of the implanted material involving separation of low molecular fractions appeared in the urine of animals during the 21st, 26th, and 54th week after introduction of these materials.

It is known that depolymerization processes may occur in plastics in the course of time as the result of "aging" and when they are used in the food industry. In this connection the question arose of the possibility of blastomogenic activity by depolymerization products when introduced into the organism per os. Study of this possibility, along with study of plastic and polymer articles intended for the food industry from the toxicologic standpoint, is necessary for the sake of more thorough characterization of such plastics.

The purpose of the present project was study of the action of polyvinyl chloride plastic on the organism of white rats after subcutaneous implantation and administration per os in the form of an aqueous extract.

Materials and Procedure

The effect of the polyvinyl chloride plastic employed in the experiment was studied with 110 white rats of no specific strain having an initial weight of 120-130 g and divided into 5 groups.

One group of 30 animals was treated daily through the drinking vessel, with an aqueous extract in the amount of 10 ml per rat.

A second group of 30 animals, which served as the control, was given only water. The treatment of the animals began with an extract prepared in advance with a plastic-water ratio of 10 cm^2/ 1 cm^3, and the contact was continued for a period of 8 months. This ratio was maintained until the end of the experiment, by the daily addition of water in an amount equaling the amount of extract consumed in treatment of the animals.

Extraction of the plastic was carried out under [Translator's Note: illegible word] conditions to accelerate the "aging".

The weight, the blood picture, and the catalase activity of the blood according to the Bach-Zubkova method were investigated in the course of the experiments as indices of the action of the substances extracted from the plastic on the organism of the animal.

The blastomogenic action of polyvinyl chloride plastics after subcutaneous implantation was studied with a group of 30 rats. The polyvinyl chloride discs, having dimensions of 1.5/2 and a thickness of 1 mm, were introduced subcutaneously on the right side of the back, under sterile conditions. The control was represented by 30 rats in which glass discs had been implanted in the same manner and another group of 20 in which no discs were implanted. The animals were observed for a period of 10 months. All the animals which died during the experiment and those killed after the experiment had been completed were analyzed from the pathomorphological standpoint.

Results and Discussion

Our experiments confirm the data in the literature regarding the blastomogenic activity of polyvinyl chloride when introduced subcutaneously. The first tumor appeared during the 11th month after implantation, the number of tumors increasing to 5 by the 14th month (see table). The percentage of blastomogenesis as calculated on the basis of the number of animals remaining alive after the sixth month following implantation (which was adopted as the minimum latency period of tumor formation) is 27.7%. The tumors ranged in size from that of a walnut to that of a goose egg, had rough surfaces, adhered to the skin and the underlying tissues, and were of solid consistency. In the case of two rats the skin covering the tumors was broken (2 tumors of a size of 0.5-1 cm, bleeding, with uneven edges). Incision of the tumors revealed the cavities in which the polyvinyl chloride discs had been situated. Microscopic examination of the histological preparation revealed 3 fibroblastic sarcomata, one polymorphous sarcoma, and one adenocarcinoma. No tumors were observed in the control animals. The group of animals treated with an aqueous extract of polyvinyl chloride resin gained less weight from the 3rd month to the end of the experiment than did the control group (see Figure 1). Investigation of the catalase activity of the blood revealed lower values in the experimental group than in the control group, the lowest values being reached in the third and 18th month after the beginning of treatment (see Figure 2). This inhibition of catalase indicates suppression of the oxidizing processes in the organism in consequence of introduction into it of substances extracted from the resin. No tumors or changes in the internal organs were observed in pathomorphological examination of the animals treated with the extract per os.

TABLE

1	2	3	4	5
Имплантат вид	Кол-во жив. в опыт	Жив. 6 мес. спустя имплант.	Кол-во животных с опухолями	% бластомогенности
6 ПВС пластинки	30	18	5	27,7
7 стекл. пластинки	30	15	-	-
8 контрол. гр.	100	20	-	-

Key: 1, Type of implantation; 2, number of animals in experiment; 3, animals alive 6 months after implantation; 4, number of animals with malignant tumors; 5, percentage of blastomogenesis; 6, PVC discs; 7, blast discs; 8, control group.

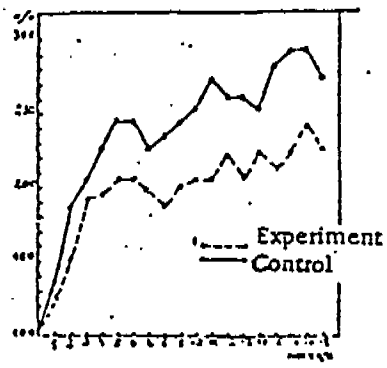

Figure 1.

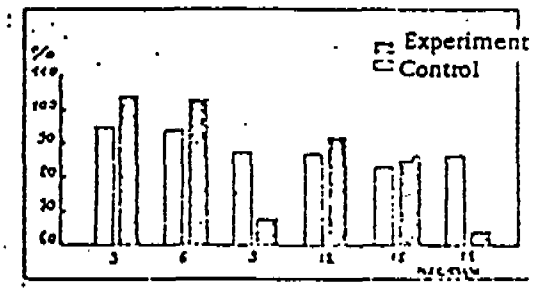

Figure 2.

Findings

Polyvinyl chloride resin introduced subcutaneously into white rats in the form of discs having the dimensions of 1.5/2.0 resulted in the formation of malignant tumors in 27.7% of the experimental animals. An aqueous extract of the same resin introduced per os over a period of 18 months had no blastomogenic effect, but the toxic action on the experimental animals is to be emphasized; it was expressed in suppression of the activity of the blood catalase and reduction in weight gain.

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1 2 61 62

The Development and Malignant	21
Transformation of Connective Tissue	22
Capsules Forming Around Implants	23
of Plastic Material -- Rats, Plastigin	24

Source (Journal, Vol., Number, Pages, Date)

1 2 61 62

Vopr. onkol. Vol. 7, No. 9, pp. 13-17,	31
1961	32

Brief Summary

1 2 10 61 62

SUMMARY:	61
	62
	63
	64

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Vopr. onkol. 7: No 9, 13-17, 1961

THE DEVELOPMENT AND MALIGNANT TRANSFORMATION OF CONNECTIVE TISSUE CAPSULES FORMING AROUND IMPLANTS OF PLASTIC MATERIAL*

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(*Received 27 August* 1960)

An aspect of great interest in recent years in the sphere of cancer research has been the study of the blastomogenic action of a number of plastic materials (cellophane, polyethylene, polyvinyl chloride, teflon, polymethylmethacrylate, polystyrene, nylon, perlon, caprone, dacron, silastic, sarane, viniene, pliofilm etc.).

While the main attention of researchers [1–5, 7–14] has been focussed on study of the properties of plastic materials (chemical structure, physical form) responsible for the causation of tumours, the features and patterns of the capsules of connective tissue developing around the implanted plastics and the

* Vopr. onkol. 7: No. 9, 13–17, 1961.

processes of their malignant transformation however have remained almost entirely without investigation.

Material and technique. Experiments were performed on white stockbred female rats weighing 100–120 g. A compressive capsule of polyvinyl chloride was affixed around the kidneys. The rats were sacrificed at 3, 10, 15, 35, 90, 195, 285, 350 and 380 days, following implantation, with 7–10 rats in each of the groups. A total of 80 animals were used. Preparations were fixed in Carnoy's fluid and mounted in paraffin. Sections were stained with haematoxylin-eosin and picrofuchsin according to Van Gieson, and impregnated by Gomori's method. Polysaccharides (glycogen, glycoproteins and mucoproteins) were demonstrated by Schiff's reagent after treatment of the sections with periodic acid with control treatment of sections with amylase, and acid polysaccharides (mucoproteids) were stained with toluidine blue and alcian blue. For their further differentiation before staining sections were treated with testicular and bacterial (streptococcal) hyaluronidase, and also with toluidine blue at various pH values. Collagenase was used for identification of scleroprotein (procollagen).

In addition, Feulgen's reaction was performed for desoxyribonucleic acid (DNA), and ribonucleic acid (RNA) was stained by Brach's method with pyronine-methyl green. Staining was accompanied by control treatment of sections with desoxyribonuclease and ribonuclease.

Results of the investigation. The initial reaction around the implant is an aseptic inflammation. Phenomena of proliferation rapidly start to predominate, the young fibroblasts being rich in ribonucleic acid. By the third day one can already observe in the capsule forming around the implant the appearance of fine argyrophilic Schick-positive fibres. The reaction of metachromasia is extremely weak during this period.

At the 10–15th day there is a capsule rich in fibroblasts and consisting of collagen fibres which stain intensely with picrofuchsin, are weak Schick-positive and do not give metachromasia at pH = 2·0, pH = 4·0 and pH = 6·0. At this stage the connective tissue capsule has a non-homogeneous structure in various areas. In some places it consists entirely of collagen fibres aligned along the implant (Fig. 1), the cell units being fairly regularly disposed and consisting of both young and mature fibroblasts. In other places the selfsame capsule has a more complex structure and consists of no less than three layers: external, middle and internal (Fig. 2). The external layer is rich in connective tissue cells. The middle layer consists of both thin and thick collagen fibres running in various directions, sometimes having the appearance of dissociated bundles. These fibres stain irregularly with picrofuchsin, and are sometimes markedly and sometimes feebly Schick-positive, without signs of metachromasia at different pH values. Sometimes one finds among the fibres in the middle layer focal accumulations of Schick-positive non-metachromatic material. In the middle layer of cells, the vessels which as a rule are not numerous are arranged irregularly. The inner layer consists of collagen fibres aligned along the implant, feebly Schick-positive, and staining red with picrofuchsin; it contains mature fibroblasts and fibrocytes located lengthwise along the fibres; there are almost no vessels in this layer. The thickness of each layer can be exceedingly variable, but the middle layer is generally thickest.

By the 30th day the capsule consists of fairly mature tissue.

After three months the collagen fibres in some parts of the capsule acquire a markedly metachromatic reaction, more pronounced at pH = 2 and staining greenish with alcian blue. The metachromasia is abolished by testicular and attenuated by streptococcal hyaluronidase. In parts of the capsule with irregular structure some of the fibres give a positive reaction of metachromasia, abolished by testicular hyaluronidase and unevenly attenuated by streptococcal

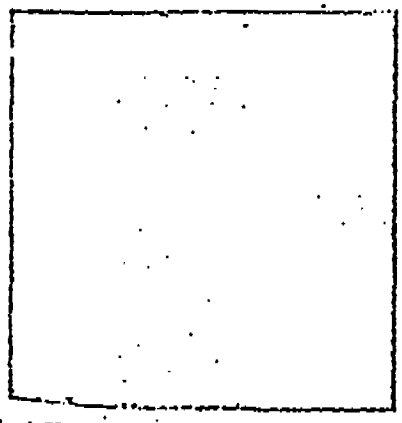

FIG. 1. The connective tissue capsule consists of ...ped collagen fibres. Van Gieson. Magn. × 130.

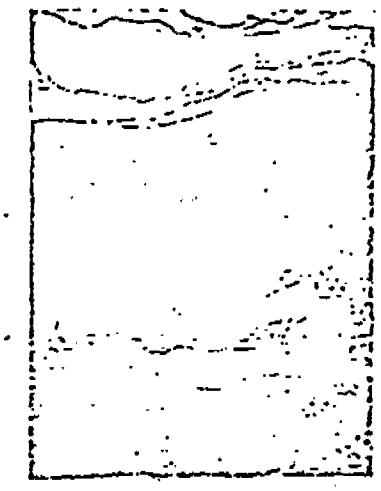

FIG. 2. Capsule of complex structure, consisting of three layers: internal, middle and external. Haematoxylin-eosin. Magn. × 130.

...aluronidase, while some are not metachromatic. In addition to the fibres, ...e metachromasia sometimes occurs in the form of irregular patches in ...ich the relation to hyaluronidase in these sites is highly variable, the ...achromasia being sometimes abolished and sometimes attenuated. The ...llagen fibres and the amorphous areas likewise stain irregularly with Schiff's ...agent, and some of them are Schick-negative. One discerns a variable rela... of the collagen fibres in areas with ordinary and with complex structure ... the enzyme collagenase. Collagenase markedly attenuates the staining with ...erofuchsin of collagen fibres in areas with complex structure, especially in ...e middle layer, and hardly affects staining with pierofuchsin of collagen ...res in areas with ordinary structure.

After three months one can observe in the capsule, in addition to hya...ized areas poor in cell elements, scattered areas of accumulation of young ...d of mature fibroblasts, as well as small cells with round hyperchromic ...cleus and mast cells. The fibroblasts contain a moderate amount of RNA, ...d sometimes their cytoplasm contains Schick-positive granules, abolished ...y amylase. Metachromasia is extremely variable in the areas of proliferation

at this stage. Proliferation of the connective tissue cells occur mainly in areas of the capsule with irregular structure and especially in the external layer.

By $6^1/_2$ months the capsule retains a non-homogeneous structure. Areas with ordinary structure are markedly hyalinized, and as a rule poor in cell elements and vessels. In some parts metachromasia is fairly well discernible. Areas with complex structure are in the main similar to those described at three months. At the sixth month one can observe in the capsule the development of focal proliferates (Fig. 3), consisting of polymorphous connective tissue cells rich in RNA, and a few mast and lymphoid cells. One can discern in the structure of these areas a marked metachromasia, most pronounced at pH = 2 and completely abolished by testicular and attenuated by streptococcal hyaluronidase. Alcian blue stains these sites a green colour which likewise is abolished by testicular and is attenuated by streptococcal hyaluronidase. Collagenase in some places completely abolishes the staining with picrofuchsin and in other places attenuates it. The Schick-reaction in the stroma of the focal proliferates is unequally pronounced. On impregnation one finds an argyrophylic framework between the cells. Some focal proliferates are clearly demarcated from the surrounding tissue, while others have an indefinite boundary.

At the 9–12th months some capsules show over their entire extent a well pronounced hyalinosis and occasional fibrocytes in the field of view. In some

Fig. 3. Formation of a focal proliferate in the sclerosed capsule. Haematoxylin-eosin. Magn. × 130.

Fig. 4. Fibrosarcoma consisting of polymorphous atypical cells. Haematoxylin-eosin. Magn. ×500.

sules however one still detects signs of proliferation of connective tissue ls and the formation of focal proliferates. In six out of 16 animal, which rvived for 9½–12½ months sarcomas were discovered (Fig. 4).

Discussion. As is seen from our data, in the period preceding the appearance the tumour in the connective tissue capsule, one observes profound anges with proliferation of cell elements. For example, by the third month re is a marked change in the polysaccharide metabolism of the connective ue capsule, and one observes a marked increase in metachromasia. The ding of most pronounced metachromasia at pH = 2 and the least at pH = 6, mplete abolition of the reaction by testicular hyaluronidase and its attenuaon by streptococcal hyaluronidase, and the results of staining with alcian ue enable one to state that in this period there occurs a rather disorderly ccumulation of acid polysaccharides (mucoproteids) both sulphuretted and hose containing carboxyl groups. The irregularity of accumulation of acid mucopolysaccharides continues right into the late stages. The reaction of metachromasia is especially intensive in focal (presarcomatous) proliferates. In the tumours the mucoproteids accumulate mainly at the periphery. It is possible that the abundant accumulation of mucoproteids is due to their imperfect elaboration and utilization. The irregular and temporally unusual accumulation of mucoproteids points to a deranged collagen formation in the connective tissue capsules forming around the implant of plastic material.

Also pointing to an abnormal collagen formation is the action of collagenase, which abolishes in some areas of the capsule the staining with picrofuchsin specific for precollagen, which suggests that these collagen fibres are of abnormal structure and different from normal fibres.

Stress must be laid on the non-homogeneous structure of the connective tissue capsules forming following implantation of the plastic material: more simple, reminiscent of the structure of the capsules around foreign bodies in some areas and complex irregular structure in others. These atypical areas are characterized by a complex structure uneven and unusual in time and amount, by accumulation of sulphuretted and non-sulphuretted acid polysaccharides (mucoproteids), by the characteristic relation of the fully formed collagen fibres to the enzyme collagenase, and by the development of unaligned collagen fibres and of extensive fields of hyalinosis. Analysis of the different stages of the development of the capsule shows that the focal proliferates develop most intensively in those very areas of the capsule which have an unusual and irregular structure. This fact emphasises the importance of the medium surrounding the cell for the onset of malignant transformation.

As seen from the results of the investigation, the development and maturation of the connective tissue capsule around the implant and the origination of the tumour go through a series of successive stages which are indissolubly linked to each other. These stages are similar to the stages of development of tumours which Shabad [6] singles out on the basis of conclusions drawn

from a large experimental material on the reproduction of tumours via the agency of various carcinogens. Four stages are mapped out. The first stage (up to 15–30 days) is characterized by the formation of granulation tissue which in some areas matures in the usual way and in other areas leads to abnormal collagen formation and the development of irregularly and complexly constructed areas of the capsule. The second stage (up to 3–6 months) is characterized by the formation of diffusely scattered accumulations of connective tissue cells and a pronounced wave of irregular accumulation of mucoproteids containing sulphate and carboxyl groups. The third stage (from 6½ months onward) is the formation of focal proliferates (presarcomas) with increase of the irregular accumulation of acid mucopolysaccharides (mucoproteids). The fourth stage (from 9½ months onward) is the formation of malignant tumours, namely sarcomas.

It must be stressed that our findings of morphological and histochemical investigation do not point to any extinction of cellular activity in a latent period at the 5–6th month, as was found by Oppenheimer *et al.* [14].

SUMMARY

1. The connective tissue capsule forming around the implant has a non-homogeneous structure.

2. Histochemical investigation indicates an abnormal course of the processes of collagen formation in the developing capsules.

3. The development around the implant of a connective tissue capsule, its further maturation and the formation of a tumour pass through a series of stages closely interlinked with each other.

Translated by L. Summerfield

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