Further Studies of Polymers as Carcinogenic Agents in Animals*

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The inception of this investigation is an example of serendipity, a word recently popularized to designate the faculty of accidentally making observations or discoveries which were not originally sought. In the present instance, a safe antihypertensive compound was being sought among the quinones to reduce the high blood pressure produced by wrapping one or both kidneys of rats with cellophane. After a couple of years, in seven of these rats malignant sarcomas had developed at the site of the wrapping. In several instances, these sarcomas had extended into the peritoneal cavity and had also metastasized. In view of this unexpected finding, a new series of experiments was begun to investigate this phenomenon in various directions. It was found that sarcomas could be induced not only by wrapping the kidney in cellophane, but alternatively by imbedding the cellophane subcutaneously in the anterior abdominal wall. By either of these methods, tumors were induced in approximately 35 per cent of the animals (10).

Further work soon showed (11, 12) that we were dealing not merely with one more carcinogen, of which hundreds were already known, but that we had chanced upon an entirely new group of carcinogenic substances, the polymer films. In 1941 Turner (13) had observed, also accidentally, that disks of Bakelite implanted subcutaneously in rats produced fibrosarcomas. Subsequent to our original report in 1948, similar results have been obtained by Druckrey and Schmähl (4, 5), Zollinger (15), Laskin, Robinson, and Weinmann (7), and Bering[1]. While, thus, there can be no doubt as to the actual facts, the interpretation of these facts as regards the mechanism or mode of action of polymers in inducing tumors is still obscure.

The present paper describes the investigations which were pursued in an effort to gain a better understanding of the processes involved in the carcinogenic action of polymers. A study was made of the effects of a number of polymers having different chemical structures. Also, because the plastics used in our earlier work were commercial products often containing plasticizers, stabilizers, traces of catalysts, a residual monomer, etc., we have imbedded a number of samples of polymers specially prepared to assure their purity. The possible carcinogenic effect of several monomers has also been investigated, and studies have been made on the degradation of polymers within the organism by means of polymers tagged with isotopic carbon.

METHODS AND RESULTS

Imbedding Procedures

In most experiments the animal chosen was the Wistar rat, but the Sherman strain was used in earlier tests, and for purposes of comparison experiments have also been done on mice of the Longacre, Paris, and C57 strains. In most experiments males were used, but, in some, females were employed with similar results. The animals were fed Purina Laboratory Chow and, occasionally, fresh carrots, and had free access to water.

The general procedure was to use small squares or circles of film, averaging 1.5 cm. in width, which had been sterilized in Zephiran[2] (1:1000 dilution of the commercial 12.8 per cent solution) for several hours, and washed in sterile saline. These were inserted subcutaneously one on each side of the abdominal wall, just ventral to the fascia.

* This investigation was supported by a research grant, No. C-1020 (C2-S), from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

[1] E. A. Bering, Jr., personal communication.

Received for publication February 24, 1955.

[2] In the early experiments, sterilisation was in 80 per cent alcohol. In a few cases films or powders have been sterilised by heat.

With few exceptions, each experiment consisted of 50 imbeddings; sometimes the same material was imbedded on both sides of 25 animals, and sometimes one form of the material was imbedded on the right side and another on the left. The animals were kept under close observation for several weeks, until the incisions were completely healed, and subsequently were examined weekly to determine the onset of tumors.

Plastics Imbedded

Most of the plastics imbedded were obtained through the kindness of various industrial firms. The designation "commercial" implies those manufactured for ordinary industrial purposes, with a more or less unknown content of nonpolymeric material. Of the "pure" forms, some have been specially made for us by the manufacturers and others prepared or purified in our own laboratory.

The following are the plastics which were imbedded:

Cellophane A.[3]—a commercial sausage casing.

Cellophane B.—the same material after extraction with alcohol for 3 days.

Cellophane C.—Cellophane B after additional extraction with benzene.

Cellophane D.—a special form employed for tissue culture work.

Cellophane O.—a Cellophane of the highest purity obtainable which was kept in formalin and washed just before imbedding.

Dacron, Kel-F, Pliofilm, Saran, Silastic, and Teflon.—all commercial products.

Nylon.—the purest form obtainable, said to contain no additives.

Polyethylene A.—a commercial film.

Polyethylene B.—specially prepared for us by the manufacturers, said to contain no additives and only a trace of catalyst.

Polyethylene HM (high molecular).—had all the low molecular weight fractions removed.

Polymethyl methacrylate A.—cast from a commercial product.

Polymethyl methacrylate D.—prepared as follows lows: freshly distilled monomer, methyl methacrylate, was sealed in an evacuated glass tube at 4×10^{-5} mm. pressure and kept at 80° C. for 2 weeks. The resulting polymer was purified by solution in methyl ethyl ketone and three reprecipitations with methanol. The film was made by casting from solutions of methyl ethyl ketone. No catalysts or additives were employed in this preparation.

[3] The designations "A," "B," "C," "D," and "O" are arbitrary and for convenience of reference only.

"Perspex."—an English preparation of polymethyl methacrylate, said to be specially pure.

Polyvinyl chloride A.—a commercial product, known to contain some additives.

Polyvinyl chloride D.—specially prepared for us by ultraviolet polymerization of vinyl chloride. Contains no plasticizers, catalysts, or other additives.

Polystyrene A.—a commercial form.

Polystyrene D.—prepared from its monomer, styrene, by a process similar to that used for polymethyl methacrylate D. It likewise contains no catalyst or additives.

Ivalon sponge and Vinyon N.—both manufactured for surgical use.

Silk film was made from natural silk fiber by dissolving it in an aqueous solution of lithium bromide, removing the latter by dialysis, and casting on glass.[4]

As will be seen from the accompanying tables, these films differed greatly both in thickness and flexibility, varying from the delicate pliable membrane of polystyrene, only 0.01 mm. thick, to the rigid disk of "Perspex," with a thickness nearly 40 times as great.

Further variations in physical form were introduced by using some of the polymers in the shape of textile fabrics (fibers), perforated films (625 holes per square inch), granules, sponges, and powders. Many of these experiments, and other modifications devised to test one theory or another, have been too recently begun for any conclusions to be drawn.

Results of Imbedding

The first demonstrable effect of imbedding a plastic film was found to be the encapsulation of the film in a sac or pocket of connective tissue of varying thickness (Fig. 1). With some films, e.g., Cellophane, the pocket wall was thick and dense and sometimes even contained calcareous areas; while with other films, such as Pliofilm or polystyrene, the pocket wall was thin and soft. This encapsulation was evident within 2–3 weeks after imbedding, and was found in animals of all ages except in cases where a tumor was induced.

The film was never adherent to the pocket but could be easily removed, leaving the pocket wall intact. Thin, pliable films were sometimes found folded or rolled, but always in a pocket, sometimes with fibrous membranes between the folds. At autopsy brittle films would occasionally be found broken, with the broken pieces either all in one pocket, or, less often, encapsulated separately.

[4] For this film we are indebted to Dr. Peter Alexander of the Chester Beatty Research Institute, London.

With perforated films and also with textiles, no definite pocket was formed, but the material was found enmeshed with connective tissue fibers which penetrated through the perforations, or between the textile threads, holding the plastic firmly in place.

In cases where tumors were produced the early stages could be palpated as a thickening or swelling around the film. The tumors grew rapidly and were usually large enough for removal (2 cm. or more in diameter) in 2 or 3 weeks after their first appearance. Since in most experiments films had been imbedded on both sides of the abdominal wall, the first tumor to appear was removed under aseptic conditions and the animal allowed to survive, in order to give an opportunity for growth of a tumor on the opposite side. Sometimes a regrowth of the first tumor occurred at or near the same site, and in three of these cases metastatic growths were found in the lung. In one or two instances in our early experiments, where tumors were allowed to grow for many months for purposes of gross demonstration, metastases occurred in axillary lymph nodes; but, in general, metastases were rarely seen.

The tumors were usually located entirely in the subcutaneous layers, but were occasionally found to have penetrated the outer layers of the muscle wall, or, very rarely, to have grown through the muscle into the peritoneal cavity. In a few instances, a tumor was found to have ulcerated outwards through the skin.

In most cases the tumor was found to surround the film more or less completely, though sometimes a portion of the film would be found projecting from the tumor, or the tumor would have grown only on one side of the film.

It is noteworthy that when a tumor developed no fibrous capsule could be found, but tumor cells were seen immediately adjacent to the plastic (Fig. 2), sometimes surrounding it on both sides, sometimes on one side only, which might be either the inner (muscle) or the outer (skin) side. If the tumor had grown on only one side of the film, then a connective tissue wall was present on the other side. Nothing definite is known as to how the connective tissue capsule disappears from the vicinity of the film. Its cells may be converted into tumor cells, or the capsule may be pushed away by tumor tissue growing between it and the film, or it may be destroyed by pressure infiltraton. In one instance where the capsule had not entirely disappeared, tumor tissue was found on the inner (film) side of the capsular remnant, as well as on its outer side and enmeshed within it. This suggested that, in this particular instance, the capsule was in the process of destruction and replacement by infiltration and pressure.

The numbers of malignant tumors obtained by these imbedding experiments are given in Tables 1 and 2.

Since these are all long-term experiments, with a latent period varying from 1 to 2 years after imbedding before the appearance of tumors, many of the experiments are still unfinished, and merely the number of tumors produced to date can be recorded. Many other experiments are in progress, but since in these no tumors have so far been produced no results can be given.

Table 1 shows the completed experiments, with the number of tumors produced and the percentage production calculated from the number of animals surviving the minimum latent period for tumor appearance. Only tumors arising around, or in direct contact with, the imbedded polymer were included as having been induced by the plastic. Any other tumors appearing in the experimental animal were interpreted as "spontaneous," and these appeared in about 2 per cent of the animals.

Including the kidney-wrapping experiments (10), our observations show 275 primary malignant tumors induced by plastics. All of these tumors were mesenchymal in origin: the large majority (over 85 per cent) were fibrosarcomas, but other types, particularly osteogenic sarcomas and rhabdomyosarcomas, were also obtained (Figs. 3a to 6). The complete list of types obtained follows:

Fibrosarcoma	235
Osteogenic sarcoma	12
Rhabdomyosarcoma	8
Mesenchymoma	6
Liposarcoma	5
Reticulum-cell sarcoma	5
Myxoma	2
Plasmocytoma	1
Histiocytoma (malignant)	1
	275

The evidence of malignancy was based on histological findings, including the frequency of mitoses, on transplantability, on occasional metastases, and on frequent local recurrence after removal of a primary tumor. The somewhat infrequent occurrence of metastases from the primary fibrosarcomas may be partly explained by the fact that the tumors were usually removed 2–3 weeks after their first appearance.

The tables show that tumors are induced whether the film is thick or thin, flexible or rigid, and that a plain film appears to induce more tumors than other forms such as perforated films, textiles, or powders. So far we have obtained no tumors

with plastics in powder form, but only one of these experiments is completed, and little significance should be attached to the observation as yet.

Controls

As control experiments we imbedded a variety of nonplastic materials, including glass coverslips, slips of wood and mica, pellets of paraffin, cotton "linters" (the fibers from which our Cellophane A was made), surgical cotton, glass cloth, and a number of metal foils. Two natural polymers, keratin (foetal nails) and a thin collagen film, were also imbedded.

Of the completed controls, as previously reported (12), linters and surgical cotton produced no tumors, but with the glass coverslip there was

TABLE 1

RESULTS OF IMBEDDING PLASTICS SUBCUTANEOUSLY IN RODENTS*

Completed Experiments

Material imbedded	Description of film: Thickness (mm.)	Description of film: Flexibility	No. rats surviving minimum latent period	Latent periods (days)	Malignant tumors: No.	Malignant tumors: Per cent
CELLOPHANE:						
A plain film	0.04	Flexible	42	495–779	15	35.7
A plain film	0.04	Flexible	35†	245–498	8	22.8
A plain film	0.04	Flexible	22‡	369	1	4.5
B plain film	0.04	Flexible	44	323–665	20	45.4
C plain film	0.04	Flexible	39	390–706	18	46.1
D plain film	0.01	Very flexible	19	423–521	3	15.8
D perforated film	0.01	Very flexible	22	504–594	4	18.2
DACRON:						
Plain film	0.02	Very flexible	41	330–693	8	19.5
Perforated film	0.02	Very flexible	42	327–651	2	4.8
Textile	0.05	Very soft	38		0	0.0
IVALON SPONGE		Soft, flexible	34	567–657	3	8.8
KEL-F:						
Plain film	0.02	Flexible	30	259–581	7	23.3
NYLON:						
Plain film	0.06	Flexible	26	441–651	7	27.0
Perforated film	0.06	Flexible	31	511–738	2	6.5
Textile	0.08	Soft	33		0	0.0
PLIOFILM:						
Plain	0.01	Soft, pliable	46	359–708	8	15.0
POLYETHYLENE:						
A plain film	0.05	Flexible	80	392–722	10	12.5
B plain film	0.02	Very soft, pliable	55	385–742	11	20.0
B plain film	0.02	Very soft, pliable	29†	343–545	3	10.3
B perforated film	0.02	Very soft, pliable	41	407–784	6	14.6
B textile	0.15	Rather stiff texture	40	497	1	2.5
HM plain film	0.07	Flexible	34	359–583	3	8.8
Powder			42		0	0.0
POLYMETHYL METHACRYLATE:						
A plain film	0.14	Rigid, brittle	20	581–658	4	20.0
POLYSTYRENE:						
A plain film	0.01	Soft, pliable	27	359–556	7	25.9
POLYVINYL CHLORIDE:						
A plain film	0.04	Soft, pliable	44	189–727	17	38.6
A perforated film	0.04	Soft, pliable	27		0	0.0
SARAN:						
Plain film	0.02	Soft, pliable	42	390–647	5	11.9
SILASTIC:						
Plain film	0.25	Rubbery	35	300–609	14	40.0
TEFLON:						
Plain film	0.02	Flexible	34	439–748	8	23.5
Perforated film	0.02	Flexible	32	526–728	6	18.7

* Except where noted to the contrary, all animals were Wistar rats.
† Albino (Longacre) mice were used.
‡ Black (C57) mice were used.

a single fibrosarcoma, appearing in the last surviving rat of fifty, 659 days after imbedding. An experiment with tin foil and one with a glass textile (fiberglas) have recently terminated, and no tumors were obtained; but the other experiments are still unfinished, and have been in progress too short a time to have any significance.

Monomers

The possibility that the carcinogenic activity of the polymers may be due to some monomer entrapped in the film, or formed as a result of polymer breakdown, cannot be disregarded. Three experiments were conducted to study the possible carcinogenic effect of monomers:

1. Ten rats were painted on the back of the neck with methyl methacrylate 3 times a week for 4 months.
2. Ten mice were painted on the back of the neck with a 50 per cent solution of styrene in benzene 3 times a week for 4 months.
3. Ten mice were painted similarly with a 1 per cent benzene solution of hexamethylene diamine, one of the components of nylon.

In none of these cases was any tumor induced, although some skin irritations resulted.

Benzoyl Peroxide

Consideration was also given to the possibility that the active carcinogen might be some residual free radical catalyst in the polymer film. To test this idea pellets containing 0.5 per cent, 2 per cent and 3 per cent benzoyl peroxide were imbedded on both sides of 30 animals. No tumors have been induced so by far these materials, but the experiment is not terminated. Pellets which were removed from the animals three or more months after imbedding contained no benzoyl peroxide. This indicates that the material had been completely absorbed, decomposed, or both, within this period. Conclusions which may be drawn from these experiments are suggested in the "Discussion."

Experiments with Tagged Polymers

To ascertain whether plastic films, although so inert, undergo any changes in the animal body, metabolic studies were made of rats imbedded with polymers tagged with C^{14}.

Radioactive polystyrene was prepared by heat polymerization of styrene-β-C^{14}($C_6H_5CH=C^{14}H_2$) in a manner analogous to that used for polystyrene D. Films of this polymer (55.0 mg.), containing 2.04×10^7 cpm/mg, were imbedded subcutaneously on both sides in the anterior abdominal wall of 25 male Wistar rats. Tagged polyethylene ($—CH_2—C^{14}H_2—$), having 8.6×10^4 cpm/mg, and polymethyl methacrylate ($—CH_2C(CH_3)—COOC^{14}H_2$), having 3.6×10^4 cpm/mg, were obtained from their manufacturers, and small pieces were imbedded in the usual manner in rats.

The different tissues and the feces were subjected to the Van Slyke wet combustion procedure (14), converted to $BaCO_3$, and counted as such. Respiratory CO_2 was trapped in 10 per cent NaOH and converted to $BaCO_3$. Urine samples were concentrated and filtered, and 1 ml. was plated directly. Background values for the latter, 28–32 cpm/

TABLE 2

RESULTS OF IMBEDDING PLASTICS SUBCUTANEOUSLY IN RODENTS

Experiments in Progress

Date imbedded	Material imbedded	Description of film: Thickness (mm.)	Description of film: Flexibility	No. malignant tumors produced up to 2/1/58	Minimum latent period (days)
10/17/52	CELLOPHANE A Perforated film	0.04	Flexible	9	553
2/18/53	CELLOPHANE O Plain film	0.03	Slightly stiff	6	413
5/5/53	PERSPEX	0.39	Rigid	3	474
1/23/53	POLYMETHYL METHACRYLATE D Plain film	0.06	Rigid	11	447
1/14/53	POLYSTYRENE D Plain film	0.04	Flexible	3	380
3/26/53	POLYVINYL CHLORIDE D Plain film	0.03	Flexible	4	533
3/6/53	SILK FILM	Irregular	Brittle	6	315
9/16/53	VINYON N Plain film	0.02	Flexible	2	440
10/1/53	POLYETHYLENE-C^{14}	0.08	Flexible	7	372
4/29/53	POLYMETHYL METHACRYLATE-C^{14}	0.10	Flexible	6	350
12/2/53	POLYSTRYENE-C^{14}	0.07	Flexible	2	268

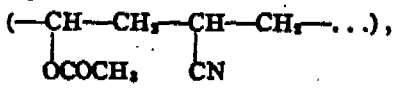
ml, were obtained with normal nonradioactive urine treated in the same manner.

With the polystyrene-imbedded rats, no radioactivity was detectable in the excreta or tissues for 21 weeks after the imbedding. At the end of this time, however, a small amount of radioactivity was found in the urine (41.3 cpm/24 hr excretion). This low level of urinary radioactivity has continued up to the present, i.e., for 40 weeks. The rats imbedded with polyethylene C^{14} began to excrete radioactive material after 26 weeks, and those imbedded with polymethyl methacrylate after 54 weeks. When the film was removed, in any of these cases, the urinary radioactivity disappeared. The urinary radioactivity cannot be due to any residual monomer in the films, since no radioactive material appeared in the urine immediately upon imbedding but only after an extended interval.

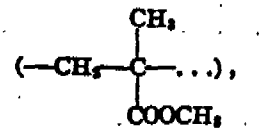
No radioactivity was detectable in the expired air, in any of the tissues, or even in the tumors which have already resulted in some cases (see Table 2). It would seem that the degradation product released from the polymer is very minute and is rapidly removed and excreted. This is the same metabolic pattern already demonstrated in previous experiments with styrene (3). The nature of the breakdown products of the polymers is still unknown; experiments to investigate these are in progress.

DISCUSSION

The carcinogenic polymers enumerated in Tables 1 and 2 differ widely in their chemical structure. The simplest one is polyethylene, which is essentially a pure paraffin, . . . —CH_2—CH_2— . . . ; there is evidence of branching of the chains (6), but otherwise it differs from paraffin only in its higher molecular weight, or chain length. Polyvinyl chloride (. . . CHCl—CH_2— . . .), Saran (—CCl_2—CH_2—CHCl—CH_2—), Teflon (—CF_2—CF_2), Vinyon N



Kel-F (—CF_2CFCl— . . .), polymethyl methacrylate



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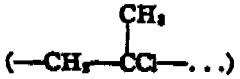
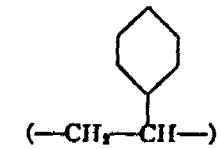
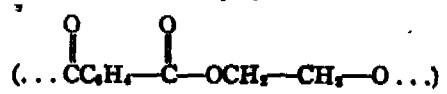


and polystyrene

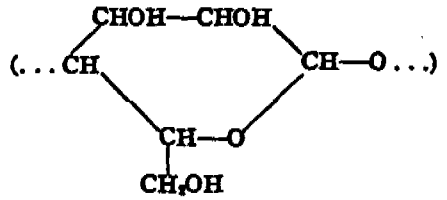


all have the polyethylene "backbone" but are substituted at various points. In short, they all are vinyl or acrylic polymers. Nylon

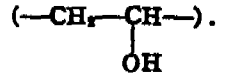
on the other hand, is a polyamide, while Dacron



is a polyester; Silastic (. . . $(CH_3)_2$—SiO . . .), is a substituted silicate, Cellophane



a polysaccharide, and Ivalon a cross-linked polyvinyl alcohol



The only common denominator in all these substances is that they are polymers, i.e., molecules of high molecular weight, containing units which repeat themselves.

The possibility that the carcinogenic agent in these experiments is not the actual plastic but some low molecular weight impurity (plasticizer, additive, or even residual monomer) was seriously considered. Pure polymers (polystyrene D, polymethyl methacrylate D, polyvinyl chloride D, and Cellophane O) which contain no additives and in which the amount of residual monomer, if any, is extremely minute, were therefore imbedded. These experiments are still incomplete, and no final percentages can be given (Table 2). However, the fact that numerous tumors have already been induced with these pure plastics does demonstrate that the primary carcinogen is the macromolecule itself, rather than any additive or impurity which may be present in the commercial film. Furthermore, the completed results with various forms of Cello-

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phane and polyethylene show that there is no relationship between the number of sarcomas produced and the degree of purity of the film. The absence of carcinogenicity in the monomers styrene, methyl methacrylate, and hexamethylene diamine rules out the possibility that monomers are the active agents, at least in these particular polymers. It therefore appears fairly certain that the carcinogenic activity of plastics is inherent in the polymer itself.

The primary difficulty in comprehending the carcinogenic action of "plastics" is the fact that they are insoluble in aqueous systems and chemically rather inert. Any interaction between them and cell components is, therefore, hard to visualize. That their activity is a result of mere mechanical irritation by friction is hardly probable, since tin foil, cotton linters, and paraffin should also produce irritation; these, however, are not carcinogenic. Moreover, there is no correlation between carcinogenicity and the stiffness or rigidity of the film, since soft, thin, pliable films often induce tumors to the same extent as the stiff rigid ones which would be expected to cause far greater mechanical irritation.

In view of this, it may be very important that our experiments with tagged molecules have shown that the polymers are degraded and metabolized in the body, at least in the rat. The amount of breakdown is extremely minute, and no metabolites have as yet been identified. This re-opens the possibility of a chemical or physico-chemical interaction between the polymer or its degradation products and some basic cell constituent of the organism. The carcinogenic activity of the polymer may thus arise in at least two possible ways. In the first place, it may be the degradation products which are carcinogenic. Since normal polymer breakdown in various aging processes proceeds via a free radical mechanism (9), it is reasonable to assume that some of the biological breakdown products may also be of a free radical nature. Free radicals are known to effect depolymerization of nucleic acids (2) and to a certain extent to produce tumors (1). A number of biological oxidations and enzymatic reactions appear to involve odd electron intermediates (8). It is possible that polymers are degraded in the presence of these biological free radicals just as they are in the presence of organic peroxides. Furthermore, the free radical fragments arising from these degradations may inhibit enzymatic free radical processes. The carcinogenic activity of polymers would thus stem from the free radical reactivity of the degradation product.

Secondly, we might visualise the creation of reactive centers in the polymer itself, as a result of the degradation. These "active centers" would then be capable of binding proteins or other basic tissue constituents and consequently might impair the metabolism of the adjacent cell.

On the basis of the long latent period it appears that the production of tumors would require the presence of free radicals in a specific area for an extended period. This may be the reason for the absence of carcinogenic activity in our imbedding experiments with benzoyl peroxide. The latter is relatively unstable and decomposes in a comparatively short period. Furthermore, tumor production may depend upon a free radical of a very specific nature and stability which is perhaps not characteristic of benzoyl peroxide.

In any case, the fact that polymers break down to some extent allows for the possibility of their chemical interaction with organic constituents and a resultant carcinogenic activity, whereas, without this evidence, one was reduced to assuming damage due to metabolic interference by mere physical obstacles, a not very satisfactory hypothesis. Of course, it may possibly be a combination of physical restriction plus chemical metabolic interference.

The extreme length of the latent period before the appearance of the tumors could be correlated with the length of time necessary for polymer breakdown and the slow rate of release of the breakdown products, since carcinogenic activity would then be a cumulative function of this degradation. If this be true, one would expect the latent period to be shortened when more "reactive" plastics, such as polymer hydroperoxides or even partially degraded polymers, are imbedded. Studies along these lines will be included in the work of this laboratory in the near future.

SUMMARY

1. Malignant tumors were induced in rodents by subcutaneously imbedding the following polymer films: Cellophane, Dacron, polyethylene, polyvinyl chloride, Silastic, Pliofilm, Nylon, polymethyl methacrylate, polystyrene, Saran, Ivalon, Kel-F, Teflon, and silk.

2. The polymer films were always found encapsulated in a pocket of connective tissue, except when a tumor was induced.

3. It does not appear that the carcinogenic activity is a result of the presence of impurities, since tumors were induced by pure polymers as well as by commercial products.

4. The monomers, styrene, methyl methacrylate, and hexamethylene diamine, were not carcinogenic in rodents when painted on the skin.

5. Studies with tagged polymers showed that they decomposed at a minute rate when left in the organism.

6. A possible mechanism by which polymers may exert their carcinogenic activity, based on the observed degradation, is suggested.

ACKNOWLEDGMENTS

We take pleasure in acknowledging our indebtedness to many who have given us help and encouragement in the course of this work, including Drs. Alexander Haddow and Peter Alexander of the Chester Beatty Research Institute, London, Dr. Arthur L. Walpole of Imperial Chemical Industries, Manchester, England, Dr. William Hueper of the National Cancer Institute, and Dr. Seymour Lieberman of Columbia University, as well as to many commercial firms and their representatives, who have so generously donated to us many of the plastic materials we needed. These include the Dow Chemical Co. and its affiliates, the DuPont Co., Goodyear Tire & Rubber Co., the Monsanto Co., and Union Carbide and Carbon Co.

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Fig. 1.—Connective tissue pocket surrounding polystyrene D film 3 months after imbedding in male Wistar rat. The film was removed before sectioning. ×330.

Fig. 2.—Pocket or cleft in a fibrosarcoma induced by polymethyl methacrylate D in 492 days, Rat No. 2922. The tumor cells completely line the cleft which contained the film (removed before sectioning). ×330.