A Comparative Study of the Effects of Imbedding Cellophane and Polystyrene Films in Rats*

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SUMMARY

The results of imbedding cellophane subcutaneously in rats were compared with previous results with polystyrene; 280 films were imbedded subcutaneously in 140 rats, which were divided into three groups and observed for tumor incidence. All animals were permitted to live out their life span.

Group I: Films were removed at monthly intervals from the enveloping pockets induced.

Group II: Pockets as well as films were similarly removed and the pockets examined histologically.

Group III: Controls; films and pockets in situ throughout.

As with polystyrene, no tumors were produced when pockets were excised, and there was a reduction in number when films alone were removed. With polystyrene, film removal before 6 months entirely precluded tumor formation, but with cellophane after film removal, even at 3 months, some tumors still appeared. The control group showed the usual tumor incidence. Histological studies showed a much greater fibroblastic activity with cellophane than with polystyrene, especially in the early months. This may be related to the shorter time of contact necessary to initiate neoplastic change (3 months instead of 6).

Other differences found with cellophane are discussed, such as its marked tendency toward calcium deposition in pockets, in tumors, and on the film itself, as well as the possible relevance to tissue activity of its permeability and its reactive surface phenomena.

It has been shown in this laboratory that polystyrene and other plastic films, imbedded subcutaneously in rats or mice, induce a percentage of malignant tumors within 1–2 years (5, 6). It has been further shown that the sheet or film form is important because it causes the formation of a fibrous encapsulating pocket, and it is in the cells lining this pocket that the carcinogenic change is initiated (7, 8). The same polymers imbedded as powders or textiles form no pockets and produce no tumors, whereas plastic sponges may or may not prove carcinogenic (7), a variation which is still under investigation.

It has been previously reported by us (4, 9) and others (3) that the pockets formed around cellophane are often thicker and denser than with other films. In the course of experiments over many years we have observed that cellophane not only induces a more active pocket than most other films but elicits a marked tendency toward a deposition of calcium in the pocket walls, in induced tumors, and on the film itself.

In view, therefore, of the characteristics of this tissue response, we decided to repeat with cellophane an experimental study previously carried out with polystyrene, in which we had investigated the relative importance of the film and the pocket at different stages of tumor induction and had correlated this with histological structure (8).

MATERIALS AND METHODS

Cellophane film (Visking 5½ H.S. sausage casing) was purified by extraction for 24 hours with hot alcohol, washed for 24 hours in frequent changes of distilled water, and subsequently cut into circular discs measuring 1.5 cm. in diameter. After sterilization for 1 hour in 1:1000 solution of Zephiran and being washed in sterile saline solution, the films were implanted subcutaneously in the abdominal walls of 140 male Wistar rats, with two imbeddings per rat, one on each side. The rats were divided into three groups, according to subsequent experimental treatment. In accordance with our usual procedure all the
animals were allowed to survive until natural death or until
tumors had developed from the imbeddings on both sides.

Group I (70 rats, 140 imbeddings): the cellophane films
were removed from the pockets by surgical technic, ten
rats being operated upon each month from the 3d to the 10th
month. The pockets were left in situ, and the animals
were allowed to survive for observation of tumors as de-
scribed above.

Group II (35 rats, 70 imbeddings): pockets as well as
films were removed at the same monthly intervals; the
pockets were examined histologically, and the rats were
kept as before for observation of tumors.

Group III (35 rats, 70 imbeddings): the animals were
kept as controls and allowed to survive for their life span
without operation, with both films and pockets retained.

RESULTS AND DISCUSSION

Table 1 compares the results obtained in this experi-
ment with those found earlier with polystyrene. In the
original polystyrene experiment as published (8) it was
noted that the number of rats in some of the groups was
small because some animals had been accidentally killed
by explosion of a steam-pipe. To supplement these
figures, therefore, we have now added data from subse-
quently comparable experiments with polystyrene. In all
cases, whether with cellophane or polystyrene, animals
that died earlier than 300 days (10 months) after im-
bedding have been entirely excluded from the statistical
results, because they failed to survive the usual minimum
latent period for tumor production.

The table shows that, without exception, with cellophane
as with polystyrene, no tumors were induced when the
pocket was removed as well as the film, demonstrating
that, with cellophane also, the carcinogenic effect is
completely localized in the pocket.

With either material, when films alone were removed,
there was a marked reduction in the number of tumors;
but a difference exists between cellophane and polystyrene

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TABLE 1

<table>
<thead>
<tr>
<th>COMPARISON OF TUMOR INCIDENCE WITH CELLOPHANE AND POLYSTYRENE AFTER REMOVAL OF FILMS OR POCKETS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REMOVAL TIME</strong> (mo.)</td>
</tr>
<tr>
<td>-----------------------</td>
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<tr>
<td></td>
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<tr>
<td>-----------------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>3-4</td>
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<tr>
<td>4-5</td>
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<tr>
<td>5-6</td>
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<tr>
<td>6-7</td>
</tr>
<tr>
<td>7-8</td>
</tr>
<tr>
<td>8-9</td>
</tr>
<tr>
<td>9-10</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
</tr>
<tr>
<td><strong>Per cent:</strong></td>
</tr>
</tbody>
</table>

**GROUP III: CONTROLS FILMS AND POCKETS IN SITU**

<table>
<thead>
<tr>
<th>TIME OF SURVIVAL AFTER IMBEDDING (DAYS)</th>
<th><strong>CELOPHANE</strong></th>
<th><strong>POLYSTYRENE</strong>*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. imbeddings</td>
<td>Tumors</td>
</tr>
<tr>
<td>266</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>300-400</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>400-500</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>500-600</td>
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<td>5</td>
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<tr>
<td>600-700</td>
<td>19</td>
<td>8</td>
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<td>700-800</td>
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<td>800-900</td>
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<tr>
<td>900-1000</td>
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<tr>
<td>1000-1100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>65</td>
<td>27</td>
</tr>
<tr>
<td><strong>Per cent:</strong></td>
<td>36%</td>
<td>35%</td>
</tr>
</tbody>
</table>

* Most of the data given here for polystyrene imbedding have already been published (8) and are repeated here for comparison with the results with cellophane. Additional data from later similar experiments with polystyrene have, however, been included in Groups II and III (polystyrene), because in the original experiment, owing to an accident, a number of rats in these categories were killed early.
when the film was removed during the early months. With polystyrene it could be seen that removal of the film up to 6 months after imbedding entirely precluded tumor formation. With cellophane, however, five tumors arose in pockets where the film had been removed during these first 6 months, although only two-thirds as many removals were involved.

It appears, therefore, that the initiation of neoplastic change took place earlier with cellophane than with polystyrene, since contact between film and pocket cells for as little as 3 months in some cases still caused final tumor production.

In view of this apparently very early initiation of neoplastic change with cellophane, as suggested by this experiment, it is somewhat surprising that the tumors induced did not actually appear earlier than did those with polystyrene.

To ascertain whether these differences in the timing of the neoplastic change might be related to differences in the cellular response, a histological survey was made comparing pockets of different ages induced by cellophane and by polystyrene, not only in this experiment but in many others over several years. Certain differences in structure and activity have been established.

The immediate response to imbedding either film is merely a typical foreign body reaction around the film, with stimulation of cellular activity and fibroblastic proliferation. The tissue rapidly becomes adapted to the form of the film, and collagen fibers are laid down in the ground substance, lying parallel to the surface of the film, which cannot be penetrated by cells or fibers (Fig. 1).

By 4–5 weeks the polystyrene pocket passes into a less active state, with fibroblasts and their nuclei lying between layers of fine collagen fibers (Fig. 2). With cellophane, at this stage, the pocket has become thicker and more active than with polystyrene (Fig. 3). This activity is not merely the usual foreign body reaction but may show extremely active fibroblasts, with large, often anaplastic, nuclei and occasional mitotic figures (Figs. 3, 4). At times the cells present a highly atypical appearance, suggestive of presarcomatous changes seen at a much later stage in pockets developing sarcomas (7, 8). These cells are usually concentrated in a band along the lumen of the pocket. Broad bundles of collagen fibers are present throughout, forming a thick enveloping sheath.

From 2 months onward the polystyrene pocket became progressively less and less active, consisting largely of collagen fibers lying parallel to the surface of the film, with inactive fibroblasts and flattened nuclei (Fig. 5). At 4–6 months the wall might be almost completely fibrous and inactive.

With cellophane, on the other hand, cellular activity continued for a much longer period, with fibroblastic proliferation continuing for many months (Fig. 6). In fact, in some pockets there might be no period of real quiescence, such as is so typical of the polystyrene pocket. This absence of a truly quiescent period with cellophane has also been reported by Vasiliev (10), who likewise noted the very early proliferation.

From 6 months onward, the quiescent polystyrene pocket may show a reawakening of activity, arising as localized foci among the cells lining the lumen (Fig. 7).

At about the same time in the cellophane pocket the areas of persistent fibroblastic activity began to show a concentration in definite foci at the lumen, although they were usually more widespread than with polystyrene (Figs. 8, 9). The end was, however, the same in both cases, when the final localized activity became the focus of tumor growth (Fig. 10).

Since our experimental results showed that, with as little as 3 months' contact, cellophane film was capable of producing tumors, we suggest that this early initiation of neoplastic change may be correlated with the early cellular proliferation noted histologically. This may be contrasted with the situation with polystyrene, whereby it has been shown that a 6 months' contact between film and pocket is necessary to initiate induction of tumors. In these pockets there was little early proliferation, and it was not until the 6-month period that presarcomatous cells began to appear in localized foci leading to tumor growth.

The higher activity of the cellophane pocket may be due to several factors which distinguish this material from polystyrene and the other plastics studied, such as polyethylene, polymethylmethacrylate, nylon, etc. We have found that these films all produce pockets similar in general to the polystyrene type, quickly becoming inactive and quiescent. Cellophane is the only one so far investigated which shows the heightened fibroblastic proliferation.

Unlike these other films, cellophane contains a large number of potentially reactive hydroxyl groups and is more hydrophilic. This allows for a certain degree of surface interaction with tissue materials. In addition, cellophane is permeable to water, salts, and low-molecular weight substances, permitting close contact between the polymer and the connective tissue constituents.

It is probably as a consequence of such chemical or physical interactions that the tissues surrounding the cellophane are more active and maintain their activity for a longer period of time than those around more inert, impermeable polymers like polystyrene.

A few experiments were carried out in an effort to elucidate the permeability factor. Attempts were made to render cellophane impermeable by backing it with an

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Occurrence of Osteogenic Sarcomas and Osseous Metaplasias</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATERIAL</td>
<td>TUMORS</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>Cellophane</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellophane (films removed)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* O.s. = osteogenic sarcoma; o.m. = osseous metaplasia; Fs. = fibrosarcoma.
impermeable material such as paraffin or polystyrene or saran. Difficulty was experienced in making the backing adhere permanently to the cellophane when imbedded, but in a few cases where this was accomplished the pocket induced no longer showed the cellular changes so characteristic of a cellophane pocket. This was true not only for the wall in contact with the coated surface but also for that exposed to the cellophane itself. This would suggest that permeability may be a factor in controlling the cellular structure of the pocket.

Still another notable difference between the action of cellophane and most other plastic films is its marked tendency to produce calcification. It is possible that this is related to the reactivity and binding capacity of the cellophane. A common observation is that when cellophane film is imbedded it may become rigid and brittle, with opaque areas showing a deposition of calcium, even after 1 month. Moreover, the pockets formed around such films often show a marked osseous metaplasia of the walls (Fig. 11), and the tumors ultimately induced include a high percentage of osteogenic sarcomas (Fig. 12).

Table 2 gives comparative figures for such ossification phenomena in tumors induced by cellophane and by polystyrene. Out of 27 tumors induced by cellophane there were seven osteogenic sarcomas and sixteen fibrosarcomas associated with osseous metaplasia. With polystyrene, out of 93 tumors induced there were only two osteogenic sarcomas and two with osseous metaplasia. In cases where the cellophane was removed at intervals between 3 and 10 months (i.e., when the time of contact with the film was reduced) out of nine tumors, one was osteogenic, and one showed osseous metaplasia.

This indicates an osseous formation of 85 per cent with cellophane as against 4.3 per cent with polystyrene, whereas the group where cellophane was removed early shows an intermediate 22 per cent.

Most of the other plastic films imbedded, like polystyrene, show only an occasional deposition of calcium. There is, however, one plastic (polyvinyl alcohol) which, in the form of a sponge (Ivalon), shows a great tendency to form deposits of calcium and phosphorus (2) in the presence of cells or fibers. It is interesting to note that polyvinyl alcohol, like cellophane, is a polymer with hydroxyl groups.

Unpublished data from this laboratory, as well as the work of Dukes and Mitchley (1), show that, with Ivalon, tumors arise in the connective tissue immediately adjacent to the surface of the implant and not in the interstices of the sponge where the deposition of calcium occurs. Tumor production appears to be independent of the tendency to calcium deposition.

Although these various tissue reactions characteristic of cellophane do not seem to determine the number of tumors, they appear to be related to the type of tumors formed and to the time necessary for the film to remain in contact with the pocket wall in order to induce them.

REFERENCES

FIG. 1.—Pocket formed around imbedded polystyrene film at 1 month, showing fine collagen fibers and generalized proliferation. Topographical. Hematoxylin and eosin (H. & E.) stain, X100.
FIG. 2.—Polystyrene pocket walls at 1 month, showing moderate generalized fibroblastic activity. H. & E. stain, X230.
FIG. 3.—Cellophane pocket walls at 1½ months, showing generalized fibroblastic activity throughout the thick wall, with a marked proliferation of cells at the lumen. H. & E. stain, X230.
FIG. 4.—Detail of Figure 3 showing active fibroblasts and one cell in anaphase. H. & E. stain, X1350.
FIG. 5.—Polystyrene pocket at 3 months, showing flattened fibroblasts of inactive pocket walls. H. & E. stain, ×325.

FIG. 6.—Cellophane pocket at 3 months, showing active cells at surface of the lumen. H. & E. stain, ×385.

FIG. 7.—Polystyrene pocket at 6 months, showing a localized patch of activity on the surface of one of the walls. H. & E. stain, ×400.

FIG. 8.—Cellophane pocket at 7 months, showing the maintained activity of the fibroblasts at the surface of the lumen. H. & E. stain, × about 300.
Fig. 9.—Focus of fibroblastic proliferation, suggesting the type that leads to sarcoma, in the wall of a 7-month pocket formed around cellophane. H. & E. stain, X470.

Fig. 10.—Fibrosarcoma located on one wall of a pocket formed around cellophane film imbedded for 23 months. The opposite wall shows an area of osseous metaplasia (o.m.). Topographical H. & E. stain, X10.

Fig. 11.—Area of pocket showing extensive osseous metaplasia. Cellophane 23½ months. H. & E. stain, X400.

Fig. 12.—Osteogenic sarcoma, induced by cellophane in 22 months. H. & E. stain, X400.
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