Virchows Arch. path. Anat. 341, 155—163 (1966)

The Effect of Varidase in Carboxymethylcellulose Jelly on Peritoneal Adhesion Formation

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Received December 2, 1965

Introduction

The management of the patient with bowel obstruction secondary to intestinal adhesions is still one of the major unsolved problems of surgery (Connolly and Smith, 1960). Moreover, the development of peritoneal adhesions following abdominal surgery may render further surgery difficult and hazardous.

Various methods for preventing experimental adhesion formation have been employed with little success, due to interference with wound healing, post-operative haemorrhage or an increase in fibroplasia. These were described in a review of the literature by Connolly and Smith (1960). More recently interest has been revived in the action of corticosteroids in adhesion prevention. Meyer and Moeschlin (1959), Rosenfeld (1959) and Schmucker and Spindler (1959), found that prednisolone esters exerted a potent, lengthy local effect when injected into body cavities such as joints. Ehlers and Grimsehl (1960) later found that prednisolone acetate resulted in a significant reduction in adhesion formation. However, Eskeland (1963) found that this mode of treatment was more effective against the formation of adhesions after primary trauma than against reformation after division. In the presence of intestinal anastomoses there was a marked tendency to haemorrhage.

Therefore, as part of an investigation into the repair of parietal peritoneum (Johnson and Whitting, 1962; Bridges and Whitting, 1964; and Bridges, Johnson and Whitting, 1965) experiments on adhesion formation were also carried out. It was noted that although after placing a blob of Varidase jelly in the area of an experimental wound covered with polyethylene sheeting adhesions still formed, they were altered histologically by the presence of peculiar "foamy" cells.

Varidase has been reported to act in the prevention of adhesions by the removal of fibrin after its formation (Connolly and Smith, 1960) and is said to have no effect on fibroblasts (see Varidase, 1964). Thus it was felt to be of interest to study these cells in more detail using an electron microscope.

Materials and Methods

Ten Wistar rats of both sexes weighing 200—300 g were used. The operative procedures were carried out observing full aseptic precautions. Under ether anaesthesia the abdominal skin was shaved and painted with a 1 in 1000 solution of Merthiolate and incised in the midline. A circular wound was made on one side of the peritoneum lining the anterior abdominal wall using a dermal punch 0.5 cm in diameter. The wound was covered with a square of polyethylene sheeting 0.058 mm thick previously sterilised by immersion in 0.1% solution of Zephiran (benzalkonium chloride) and washed in sterile isotonic saline. The plastic

was sutured over the wound at the four corners with 4 atraumatic ophthalmic sutures and a large blob of Varidase jelly (streptokinase and streptodornase in carboxymethylcellulose jelly) applied to the area (see Fig. 1). The abdomen was then closed in layers and compound benzoin tincture U.S.P. applied to the skin incision. Five animals were operated on in this way, and in a further five animals no Varidase was applied. The latter group were used as controls.

Five days after operation the animals were killed by ether inhalation and the wound areas were examined macroscopically. The wound areas were excised, pinned to small cork boards to prevent shrinkage and fixed in alcoholic formalin, embedded in paraffin and sectioned at 7μ.

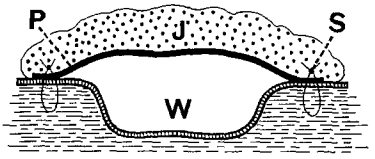

Fig. 1. Diagrammatic representation of the plastic sheeting sutured over the wound area. *W* Wound, *J* Varidase and C.M.C. jelly, *S* suture, *P* Polyethylene sheeting

For electron microscopy small pieces from the adhesions were placed in ice-cold, 1% osmium tetroxide, buffered with Veronal, to which sucrose had been added. After fixation for 1 hour the blocks were embedded in Epon or Maraglass.

Results

Macroscopic Findings

The five control animals all exhibited dense peritoneal adhesions to the wound area. In the group treated with Varidase jelly three animals showed dense adhesions and two exhibited filmy adhesions.

Light Microscopy

In the control animals the polyethylene sheeting is encysted by omental adhesions to the wound area. There is a marked fibroblastic reaction in the wound beneath the sheet but no cells of mesothelial appearance are visible. Adjacent to the points of adhesion the omentum shows a thickening of the normally thin surface layer of cells, and at the point of adhesions to the margin of the wounds there is marked fibroplasia. In these areas numerous mast cells are present and some show signs of partial degranulation.

In the Varidase treated animals the adhesions and exudate at the margins of the wound contain large numbers of irregularly shaped cells with a foamy appearance (see Fig. 2). These cells are present in large clumps scattered throughout the omentum and also at the omental margins adjacent to the wounds. Sections stained with haematoxylin and eosin reveal large cells with a central or eccentric darkly stained nucleus which is rounded and contains two nucleoli (see Fig. 3). The cytoplasm is "foamy" in appearance and contains some granules.

In sections stained with the techniques for demonstrating mast cells the cytoplasm of the "foamy" cells exhibits a pale metachromasia. Mast cells are present in large numbers and some show partial degranulations. In areas in which mast cells are visible some of the "foamy" cells contain a few granules which are metachromatic.

The material contained in these cells is similar in appearance and staining characteristics to Varidase jelly which exhibits a pale metachromasia and appears to have been phagocytosed by the "foamy" cells. In areas adjacent to the wound,

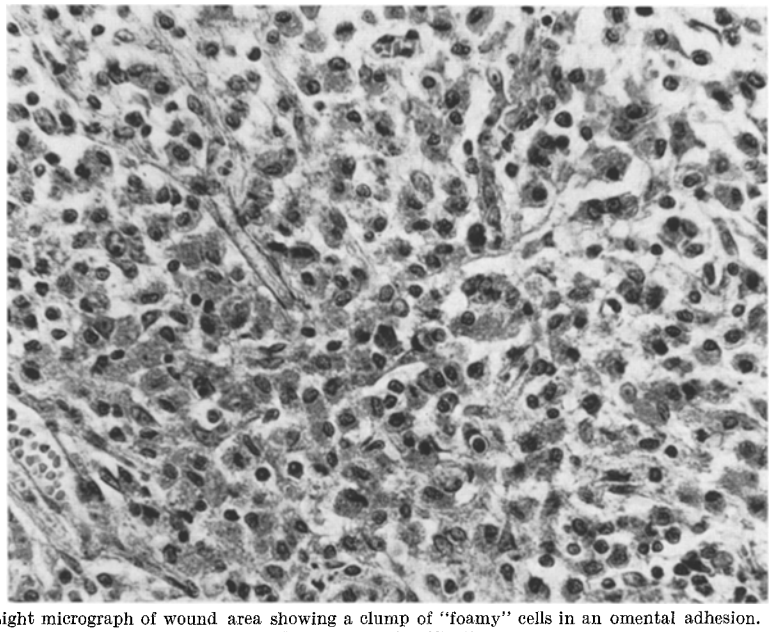

Fig. 2. Light micrograph of wound area showing a clump of "foamy" cells in an omental adhesion. Toluidine blue and ether 181. Magnification: 350×

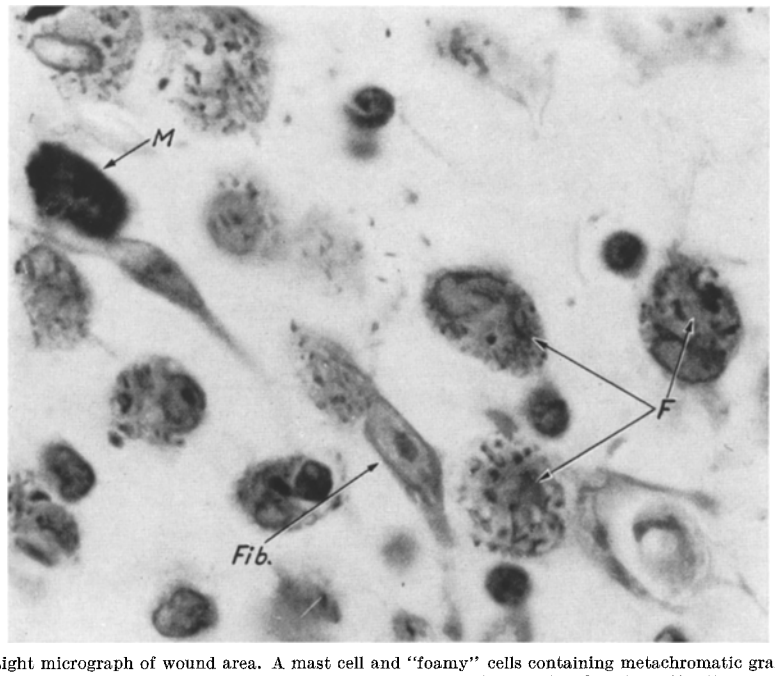

Fig. 3. Light micrograph of wound area. A mast cell and "foamy" cells containing metachromatic granules can be seen. Toluidine blue and ether 181. *M* mast cell, *F* foamy cell, *Fib* fibroblast. Magnification: 1200×

areas of metachromatic material were seen which is identical with that contained in the "foamy" cells. Fibroblasts and macrophages of normal appearance are also present.

Electron-Microscopic Appearances — Controls

In the control animals the adhesions have a covering layer of mesothelial cells. These cells appear flattened and have microvilli on their free surface. In addition to the usual organelles these cells contain numerous small vesicles. The cells rest

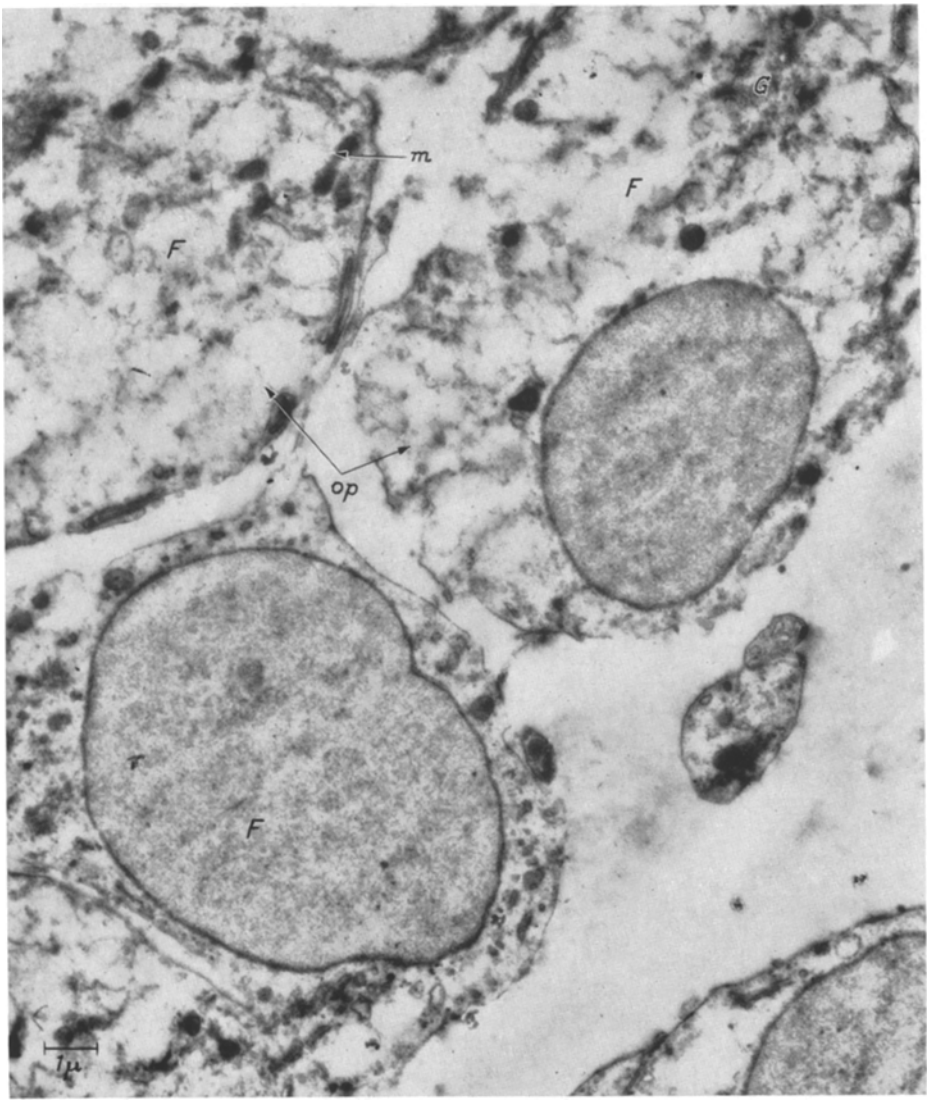

Fig. 4. A low power electron micrograph of part of an adhesion from a Varidase treated animal. Numerous "foamy" cells can be seen. *F* "foamy" cell, *G* Golgi zone, *m* mitochondria, *op* opaque substance. Magnification: 7,800×

on a thin basement membrane, which is often associated with collagen fibres. Fibroblasts are seen close to the mesothelial cells and also scattered throughout the adhesion. The fibroblasts typically appear elongated, often with indented nuclei. They can usually be recognised by the extensive amounts of ergastoplasm and scattered ribosomes in the cytoplasm. Mitochondria, a Golgi zone, many small

vesicles and occasional myelin type figures can also be seen. Collagen fibres are often in very close association with the plasma membrane of these cells.

Macrophages are also present characterised by their larger size and more rounded appearance although they are sometimes difficult to distinguish from

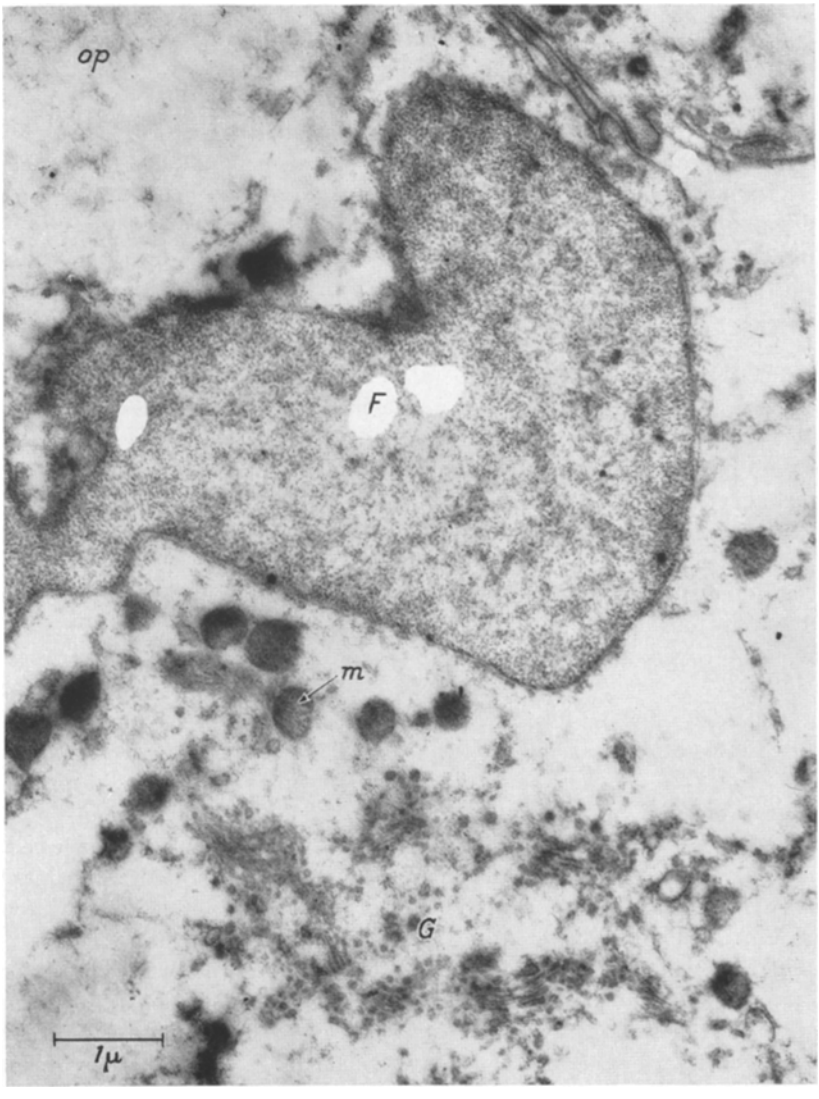

Fig. 5. A higher power electron micrograph of part of a "foamy" cell showing the opaque substance and Golgi zone. *F* "foamy" cell, *op* opaque substance, *G* Golgi zone, *m* mitochondria. Magnification: 14.500×

the fibroblasts. They contain relatively small amounts of ergastoplasm and few scattered ribosomes. Numerous mitochondria, small vesicles, large vacuoles and lysosomes are present.

Many fat cells are seen in the adhesions and also occasionally mast cells and polymorphonuclear leucocytes.

Electron-Microscopic Appearances — Varidase Treated

In the animals treated with Varidase one cell type predominates — the "foamy" cell. These cells are often large, round and appear distended with an electron dense, opaque substance (Fig. 4). The cells are usually grossly distended but sometimes cells with only a few vesicles are seen. In these cases the cells are more elongated and contain quantities of ergastoplasm giving them the appearance of fibroblasts. Due to the gross distension it is difficult to see if the opaque material is contained in a membrane. Occasional cells with the cisternae of the ergastoplasm distended are seen but in the larger cells the membranes break down and the material appears to coalesce. The nucleus is usually rounded but indentations are sometimes seen. Mitochondria are present and a prominent Golgi zone can be seen in the cell near to the nucleus (Fig. 5).

In addition to the "foamy" cells, numerous fat cells are present. No normal looking fibroblasts nor macrophages are seen in the E.M. sections.

Discussion

Streptococcal metabolites have been used on several occasions for the prevention of experimental adhesions but with conflicting results (see CONNOLLY and SMITH, 1960). For example, WRIGHT et al. (1950) using streptokinase and streptodornase prevented traumatically induced adhesions in rabbits but not in dogs. Discrepancies like these can be explained by the known species differences that occur in adhesion formation. BRIDGES et al. (1965) showed that experimental adhesions were very uncommon in the rabbit, but that in the rat they always occurred following the infliction of a wound which was covered with polyethylene. For these reasons, therefore, the rat was used in our series of experiments and the wounds were covered with polyethylene so that we could be certain of producing adhesions.

LUTTWAK et al. (1954) found, that in the rat, streptokinase and streptodornase prevented talc granulomas and intestinal adhesions with some success. In the dog, SHERRY et al. (1955) found that intravenous streptokinase prevented or modified adhesion formation. LUDEKE (1961) suggests that the use of fibrinolytic agents postoperatively is useless and that any action to prevent adhesions must be taken at the time of operation. More recently JEWETT et al. (1965) were unable to demonstrate any significant prophylactic or therapeutic effect using intraperitoneal and intravenous fibrinolytic agents in rabbits, rats, and dogs. In our experiments Varidase (Streptokinase — streptodornase) was used directly on the wound area by applying it in C.M.C. jelly (carboxymethylcellulose). Although the adhesions formed were filmy in two animals, the remainder had firm adhesions but the histological structure of the adhesions was altered by the presence of large numbers of "foamy" cells.

Varidase (LEDERLE) is a mixture of streptokinase and streptodornase and is usually considered to prevent adhesions by the removal of fibrin after its formation (CONNOLLY and SMITH, 1960). Streptokinase acts with plasma to produce an active proteolytic enzyme, plasmin, which can then lyse fibrin. Streptodornase is a group of enzymes which act to liquefy desoxyribonucleo-proteins which are constituents of purulent exudates (Extra Pharmacopoeia 1952). They are thought to work by liquefaction and have no direct action on fibroblasts themselves

(VARIDASE, 1964). However, in the present experiments, the vehicle used to contain the Varidase was C.M.C. jelly (carboxymethylcellulose). This substance has been shown to produce an increase in the number of mast cells in rats when used as a suspending medium (ROMANI, 1953). However, SELYE (1965) in a review of the mast cells thought that the cells described by ROMANI were histiocytes containing metachromatic carboxymethylcellulose granules. JASMIN and BOIS (1961) followed the distribution of C.M.C. injected into rats by histochemical means and found that the phagocytosed intercellular granules of C.M.C. stained metachromatically. BURTON (1963) studying the phenomenon of phagocytosis of mast cell granules by connective tissue cells cinephotomicrographically, found that cultures of rat connective tissue absorbed C.M.C. which was included in the culture medium giving rise to cells which in the early stages resembled mast cells morphologically but did not stain metachromatically.

The "foamy" cells seen in our experiments contained metachromatic material and some granules, although the metachromasia in sections stained with toluidine blue in Ether 181 was paler than that exhibited by unruptured mast cells present in the omentum. These findings would correspond to those of SELYE (1955) who described cells similar to mast cells in the adrenal medulla following prolonged treatment with C.M.C. He suggested that these cells were probably developed by the phagocytosis of metachromatically stained material. As a result of the presence of the "foamy" cells and also of areas of metachromatic material around the wound which had not been phagocytosed in these experiments, smears of C.M.C. jelly were stained with toluidine blue in Ether 181 and when examined exhibited a similar pale metachromasia. This agreed with the findings of GRAUMANN (1957) who obtained a positive metachromasia with C.M.C.

It appears probable that the C.M.C. jelly is ingested by cells present in the wound area and also by cells of the omental adhesion. In some cells the distension is considerable so that the cells might no longer be able to carry out their normal functions (cf. the light cells of the thyroid gland: YOUNG and LEBLOND, 1963). This is supported by BURTON's experiments (1963) who found that after six days the cells containing phagocytosed material showed symptoms of degeneration, their cytoplasm being overloaded with inclusions.

It is difficult to assess whether the formation and structure of the adhesions was affected by the presence of the Varidase. In two animals the adhesions were filmy which suggests that the Varidase may have exerted some lysing action on the fibrinous exudate, but any effects produced were probably overshadowed by the production of the large numbers of "foamy" cells. It seems, therefore, that the use of Varidase in C.M.C. jelly would be ineffective in preventing adhesions as the C.M.C. acts by producing cells, which according to BURTON (1963) become inactive and die at about ten days. These areas of necrotic cells might then lead to future adhesion formation by promoting fibroplasia.

Summary

Experiments are described in which Varidase in carboxymethylcellulose jelly was used in an attempt to prevent peritoneal adhesion formation. This appeared to have no effect on adhesion formation although light and electron microscopy

revealed the presence of "foamy" cells in the adhesions and wound areas. These cells were probably formed by the phagocytosis of carboxymethylcellulose jelly by connective tissue cells.

Die Wirkung von Varidase in Carboxymethylcellulose-Gelee auf die Bildung peritonealer Adhäsionen

Zusammenfassung

Bei entsprechenden Versuchen an Ratten gelang es nicht, durch Anwendung von Varidase in Carboxymethylcellulose-Gelee die Bildung von peritonealen Adhäsionen zu verhindern, obwohl licht- und elektronenmikroskopisch „Schaumzellen" in den Adhäsionen und in den Wundgebieten nachweisbar waren. Diese Zellen verdanken ihre Entstehung wahrscheinlich der Phagocytose von Carboxymethylcellulose durch Bindegewebszellen.

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