An Experimental Study of the Effects of a Plastic Adhesive, Methyl 2-Cyanoacrylate Monomer (M 2 C-1) in Various Tissues*

J. Dutton, M.B., F.R.C.S., and P. O. Yates, M.D.

Departments of Neurosurgery and Pathology, Manchester Royal Infirmary, and the University of Manchester, England

M 2 C-1† has been under investigation as a tissue adhesive for several years. It is a clear, mobile liquid which forms strong bands when a thin film is lightly pressed between two surfaces. No heat, excessive pressure, addition of solvent, or catalyst is required for polymerisation. Bonding occurs through a mechanism of anionic polymerisation aided by traces of water; the adhesive acts by its molecular attraction (specific adhesion) and by the interlocking of set adhesive on irregular surfaces (mechanical adhesion).

Since 1960, a number of investigators have studied the physical and clinical properties of this material, and breaches in the continuity of a variety of tissues that have been repaired with it. Seventy references had been collected and reviewed up to November 1964. Scrutiny of these papers, which are primarily laboratory and animal studies with only a few clinical reports, suggests that most of the authors are favourably impressed with M 2 C-1 as a glue; to date only 3 papers have been noted where caution was advised in its use. Not all the references are to the use of the pure monomer; various allied preparations containing inhibitors and plasticisers have been used, but today are not recommended for biological use.

Ten years ago, a method of investing intracranial aneurysms with self-curing methyl methacrylate was described by one of the present authors. In 1958, a report of 15 cases was published and recently, further information on 30 cases was presented. Amongst other theoretical criticisms of the use of methyl methacrylate was that, in its polymerised state, it was hydrophobic and thus never adhered to the tissues. There was a possibility (never encountered in practice) that in cases of total investment, rupture of the aneurysmal sac might result in 'tamponade' of the sac and parent vessel within the acrylic shell. In an endeavour to overcome this objection and achieve a 'mend' between the sac wall and the investment, samples of M 2 C-1 were eventually obtained from Ethicon in 1963. The purpose of this paper is to present the results of experimental application of minute quantities of this monomer to a variety of tissues.

Method

Male and female cats whose weights varied from 2 to 2½ kilogrammes were used. All were anaesthetised with Pentobarbitone sodium (Nembutal) 35 mg./kg. of body weight. During the early stages of the study, M 2 C-1 was used from a 15 cc. bottle. More recently, sterile 1 ml. tubes have been used; this eliminated our original concern regarding deterioration of unused material in the bulk containers. Aseptic technique was maintained throughout the procedure.

Group 1(a). Surface of the brain. Thirteen cats were subjected to bilateral craniectomy exposing the lateral supra and ecto sylvian gyri. The dura mater was incised; the resultant defect was closed by a free fascial graft, coated with a small quantity of M 2 C-1 and light pressure applied. Saline was used on the contralateral side as a control.

Editorial Note. The following summary may be of help in sorting out the nomenclature involved.

1. Methyl 2-cyanoacrylate. The basic adhesive chemical involved.
2. Eastman 910 adhesive. A general bonding agent purchasable at hardware stores. It contains (1) plus several additives including the alleged carcinogen methyl methacrylate.
3. Eastman 910 monomer. This is pure (1) with no additive; it bonds very fast (± 5 sec.) and has not been sterilized.
4. Eastman 910 monomer (clinical grade) M 2 C-1. This is basically (3) that has been sterilized; it has also been inhibited by SO₂ so that it bonds more slowly (50 sec.). The Food and Drug Administration is allowing limited clinical trial of this product.

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The wounds were closed by single layer skin sutures. The animals were sacrificed at periods varying from 3 days to 8 weeks. The brain and dura in relation to the craniectomy were removed in one piece and fixed in 10 per cent formalin for 2 weeks. After brain cutting, tissue blocks were cut, embedded in paraffin and stained routinely with Weigert's haematoxylin and eosin for cell detail, methasol fast blue to show myelin, phosphotungstic acid haematoxylin or Holzer's stain to show gliosis, and Gros-Bielschowsky silver stain to show neurofibrils.

Results. All animals survived the experimental procedure. There was no evidence of infection, epilepsy, cerebrospinal fluid leak, or neurological deficit. At brain cutting the fascial graft was adherent to the dura, and the brain was adherent at the gap in dural continuity (Fig. 1). Coronal sections through graft, dura and brain were made and processed as above.

In most cases the graft was surviving; in places between it and the dura there were pools of refractile M 2 C-1, always closely surrounded by a zone of fibrin. Around the fibrin, a zone of nuclear debris and fragmenting polymorphs was present (Fig. 2); these changes persisted for at least 7 weeks. Beyond this layer a granulomatous healing reaction with macrophages, fibroblasts, new collagen and capillaries was found which in the later specimens became mature fibrous tissue.

At the dural gap, the new scar tissue extended through and was adherent to the pia arachnoid. Here the surface layers of the cortex showed a small amount of reactive gliosis (Fig. 3), but no loss of neurones (Fig. 1), and only occasional nerve cells showed ischaemic changes. In very early specimens there was some oedema of the outer 2 layers of the cortex, but this did not persist.

Examination of the control slides revealed relatively minor changes with some later fibrosis of dura and arachnoid.

Group 1(b). Within the brain. In 5 other cats, after making a breach in dural continuity and incising the arachnoid, a track approximately 2 cm. long was made vertically into the brain with a wooden applicator stick, the end being smoothly pointed. On one side monomer was applied, while on the other side normal saline was used as a control. These animals were sacrificed at periods varying from 2 to 8 weeks, and the brains fixed and sectioned.

Results. All specimens showed brisk polymorphonuclear reaction along both tracks, but on the side of M 2 C-1 application there was neuronal death extending for a distance beyond the track which showed inflammatory reaction. Later specimens showed microglial macrophage collections and reactive gliosis along the track which extended through pia arachnoid to form an adhesion
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**Fig. 3.** Surface of brain showing sub-pial gliosis merging with scar tissue which surrounds polymer adhesive (visible at top right). H. & E.; ×250.

**Fig. 4.** Nerve bundle showing some break-up of myelin and an exudate beneath the membrane. H. & E.; ×300.

**Fig. 5.** Down the side there is a strip of refractile glue to the right of which is an anuclear necrotic zone involving part of a small artery, and at the top a larger vein. Three nerve twigs at the bottom are destroyed; the other two are involved in a granulomatous inflammatory reaction. H. & E.; ×40.

with the dura. The control track merely showed gliosis.

**Group 2. Peripheral Nerves.** The saphenous nerves of both hind limbs of 30 cats were exposed. All nerves were carefully dissected free, care being taken to avoid damaging small vessels, and a segment of nerve 1 cm. long was coated with a small quantity of M 2 C-1. On the control side, saline was applied. All wounds were sutured. All animals remained well during survival and showed no obvious neurological deficit. At 2, 4, 6 and 8 weeks these animals were sacrificed, the nerves removed, fixed in 10 per cent formalin for 2 weeks, embedded, sectioned, and stained.

**Results.** All specimens of nerves treated with M 2 C-1 showed increased adhesion and fibrous reaction around the treated area; some showed a light brown discolouration.

In the majority of specimens, the nerve bundles were virtually normal when compared with the controls. However, in many of these there was no
adhesive found in direct contact with the perineurium. Where contact had been established, there usually was evidence of damage. The most severe lesions took the form of a breakdown of myelin and swelling of axons which later went on to macrophagic activity and fibrosis (Fig. 4). The most severe damage with total fibrosis was found to involve those nerves which passed through, or close by, florid granulomatous reactions (Fig. 5). Occasionally, however, nerve bundles were found in juxtaposition with fibrin-enclosed polymer but without evidence of damage (Fig. 6). Few showed any thrombosis of small vessels.

Nerves from control operations showed minimal changes only.

Group 3. Arteries and veins. In 13 cats, M 2 C-1 was applied to the carotid artery on one side, and in 20 cats to the saphenous artery. In all instances the contralateral vessels were also dissected and coated with normal saline. In some instances, linear breaches in continuity were produced, and fascial grafts applied over M 2 C-1 glue to secure haemostasis. The animals were sacrificed at periods varying from 2 to 8 weeks. During these periods, all animals appeared well and all wounds healed satisfactorily.

Results. Eleven of the 13 carotid artery specimens, and 10 of the 20 saphenous artery specimens, showed changes of severe degree. Eight carotid and 9 saphenous arteries showed partial or total medial muscle necrosis of varying radial and circumferential extent. In all vessels the endothelium was intact; it had apparently been reformed in one case. There was intimal thickening in some damaged arteries beneath the zone of medial necrosis (Fig. 7). The internal and medial elastic laminae and the collagen appeared normal (Fig. 7). The cell death always appeared to be related to nearby pools of refractile M 2 C-1, and the extent of involvement of the arterial wall depended on how closely it was invested by the glue (Fig. 8). Occasionally as much as three-quarters of the circumference was necrotic for the full thickness of media and adventitia. The glue was always enclosed in a lake of fibrin as in other sites, and this in turn was surrounded by poly-

![Fig. 6. Nerve twig in close proximity to adhesive but showing no abnormality of axons or myelin. H. & E.; X300.](image)

![Fig. 7. Part of an artery, the media of which is almost entirely replaced by collagen. This shows preservation of elastic and intimal thickening as part of the healing process. Weigert's elastic stain; X90.](image)

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morphs, some of which were necrotic. In specimens from longer survivors the polymorphonuclear reaction to the M 2 C-1 was still marked, but by this time granulation tissue and fibrosis were well advanced.

The damaged sector of the arterial wall was replaced by new fibrous tissue. No evidence of regeneration of smooth muscle could be found. One case at 6 weeks showed calcification of the damaged area which extended to 50 per cent of the medial circumference (Fig. 9).

Thrombosis was seen in only 2 arteries; one carotid showed a patch of mural thrombus beneath a necrotic sector (Fig. 8); one small (300 μ) saphenous artery had been occluded by thrombus with organisation and recanalisation (Fig. 10).

In the 2 carotid artery specimens where no changes were evident, the M 2 C-1 was lying outside the carotid sheath, and it was thought that this lack of physical contact possibly accounted for the lack of deleterious effect in these and other non-affected vessels. The accompanying veins were studied in 11 cases and 9 of these showed changes comparable to those noted in relation to the arteries, that is, death of smooth muscle cells and replacement by fibrous tissue.

**Discussion**

There is no doubt from these and earlier experimental studies that M 2 C-1 is a very effective glue for any 2 surfaces, whether these be tissues of the body or non-living materials. With the latter the surfaces which are glued together may be expected to remain unchanged, and thus the union is a permanent one. The situation is quite different.

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Fig. 8. An artery showing necrosis of the media for about one-third of its circumference on the side nearest to the glue (visible in the bottom left-hand corner having a vertical rippled appearance). H. & E.; X120.

Fig. 9. Artery showing replacement of half the media by a calcified and bony strip. H. & E.; X70.
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Fig. 10. A small saphenous artery (near a strip of glue) occluded by thrombus which is almost completely organised. H. & E.; ×160.

when biologically active surfaces are involved. The first reaction to the presence of a layer of this glue appears to be the formation around it of a pool of fibrin, perhaps as a response to the negative surface charge activity which is so important for its adhesive properties. Once this has occurred, and certainly within a few hours, the tissue on both surfaces is separated from the glue by a layer of fibrin and the union is only as effective as the strength of a fibrin film.

Furthermore the fibrin itself soon becomes surrounded by polymorphonuclear cells which have a marked proteolytic activity, and therefore tend to separate the glue and fibrin layers from underlying surfaces. Nevertheless the polymorphs and macrophages which accumulate also seem unable to complete the removal of the fibrin within a reasonable period, perhaps because further fibrin is continuously laid down or because of some noxious effect of the nearby polymerised glue.

The granulation tissue, which represents the reaction through which healing and scarring occurred, varied in its exuberance from animal to animal. It seemed likely that this was affected less by the quantity of glue which was used than by normal variations in host response.

In some of the original papers on the use of this glue it was stressed that the use of non-sterilised material never resulted in sepsis, and bacteriological examination revealed no organisms in cultures of the material. It was concluded that the glue was self-sterilising. We suggest that whatever noxious property kills all bacteria may also be responsible for the damage to tissues which was found in the present experiments. Control mock operations showed that these changes are not the result of technical trauma or infection. Death of cortical neurones and smooth muscle cells of the arterial media was noted up to a distance of about 1200μ from the glue. It is possible that other types of cell are also destroyed but because they are mostly migratory and replaceable, the loss will not be detectable after a few hours. This suggests that the damaging effect is a transient one such as would be produced by the heat of polymerisation or the sudden release of a solvent or inhibitor into the tissue fluids. The material which we used for these experiments was stated to be pure monomer, the implication being that no additives are present. If it is heat which destroys nearby cells, then there should be a quantitative relationship with the amount of glue used. We could not be certain on this point, for it is very difficult to estimate the quantities that are being used when applying glue in the depths of a
wound; minimal amounts were always used, the total never being greater than 1 or 2 drops.

Some evidence that heat is not the principal damaging agent is provided by the fact that certain membranes appear to act as a protective barrier. Thus the pia arachnoid and the epineurium of peripheral nerves seemed to have this property. No damage comparable to that found after surgical diathermy to non-vital structures such as collagen or elastin could be seen.

In none of our experiments was there any evidence as to how the glue may finally be dealt with by the body. The material was always enclosed in fibrin, even after several weeks; the fibrin seemed to act as a barrier preventing access by macrophages or giant cells, which were never found to contain fragments of glue. It may be that the strong negative electrostatic charge at the surface of the polymer encourages continuous local fibrin deposition, as in the experiments reported by Bangham.¹

In spite of the total cell death of whole sectors of the walls of blood vessels, thrombosis was not a common sequel; only 2 arteries showed any, and the one which had been completely occluded by thrombus was very small. Nevertheless one could not recommend the use of M 2 C-1 as a glue for small blood vessels or as a substance for ensheathing cerebral aneurysms. Loss of medial muscle and the related arterial elastic response might encourage further aneurysm formation. The title “Physiological Tissue Adhesive” which is applied to M 2 C-1 glue appears to be somewhat misleading. We feel that the neurosurgical use of this material should be restricted to the fixation of grafts, especially when closing dural and bony defects during radical surgery for neoplasia.

Summary

1. M 2 C-1 was applied to the dura and surface of the brains of 13 cats; nine showed a brisk polymorphonuclear reaction and later granulomatous healing. The underlying brain showed little structural or functional change, and it appeared that the pia and arachnoid mater acted as a barrier to the effects of M 2 C-1. The controls were always unremarkable.

2. In 5 cats M 2 C-1 was instilled into vertical tracks made into the brain substance. Death of neurones and a marked glial response were noted.

3. In 30 cats, the saphenous nerves were coated with M 2 C-1. Eight specimens showed perineural inflammation, and neurilemmal and axonal damage occurred in some cases.

4. Thirteen carotid and 20 saphenous arteries were coated with M 2 C-1; 11 of the former and 10 of the latter showed changes varying from total medial necrosis to death of smooth muscle confined to a sector nearest to the glue. Thrombosis occurred in only 2 arteries.

5. Almost all specimens showed a brisk inflammatory or granulomatous response to the glue. For several weeks the material is surrounded by a lake of fibrin which rapidly separates the film of glue from the tissues. The strength of the union after an initial short interval cannot therefore remain greater than that of fibrin itself.

References


