NEW ABSORBABLE HEMOSTATIC BONE WAX

EXPERIMENTAL AND CLINICAL STUDIES*

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IN RECENT YEARS a number of new absorbable hemostatic materials have been developed. These were studied primarily for use in neurosurgery but have now been widely employed in general surgery as well as genito-urinary, orthopedic and gynecologic surgery and even in obstetrics. One of us (V. K. F.) had the opportunity to work, under OSRD contract, on oxidized cellulose, *i.e.,* absorbable gauze and cotton. The specific styptic action of these agents, apart from their hemostatic property has been obvious in clinical use.

It was suggested by Henderson,[6] therefore, that advantage might be taken of this specific hemostatic action in conjunction with another kind of hemostatic packing also studied primarily by neurosurgeons, *i.e.* bone wax. Horsley's note in the British Medical Journal in 1892[8] described the formula of bone wax as seven parts beeswax, one part almond oils, and salicylic acid 1 per cent. This had the desired physical properties, was kept in stoppered bottles and sterilized by boiling. The use of this material for control of bleeding from bone became a standard neurosurgical technic and was also used when indicated in other fields of surgery, such as chest surgery, for example, when the sternum is split longitudinally.

Ordinary bone wax is effective by virtue of its tamponade action, but it has no inherent hemostatic quality. In addition, it is not soluble in the body fluids and thus remains at the site of implantation for long periods of time. That portion of it which eventually is removed is probably carried away through the action of phagocytic cells. As a result, the wax acts as a foreign body, tending to promote infection and in itself causing a low grade inflammatory reaction in the tissues about the site of implantation.

Oxidized cellulose and the new water soluble ointment bases offered the possibility of developing a substance which had the same physical properties as the beeswax mixture of Horsley and also was specifically hemostatic and absorbable. At Henderson's suggestion,[6] some of the polyethylene glycols of high molecular weight were chosen as the base. A hemostatic absorbable substance has now been produced and is the subject of this study.

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COMPOSITION AND PROPERTIES OF THE NEW ABSORBABLE, HEMOSTATIC BONE WAX

The material is dispensed already sterilized by autoclave, wrapped in scored tinfoil and ready for use in sealed glass tubes of the type familiar to all who have used catgut sutures. It has a consistency somewhat firmer than petrolatum but is definitely less firm and more malleable than ordinary bone wax. Since it melts near body temperature, handling the material for any length of time causes it to become softer. Prolonged handling will eventually cause it to liquify. The material has a uniform, dark brown color.

Because of its greater malleability and softer consistency, this material is more easily applied to the bleeding crevices and openings in bone than the ordinary type of wax. In addition, it does not have the tendency to crumble into small particles, and after it has been applied adheres much more tenaciously to the bony structures. It adheres to rubber gloves somewhat more than ordinary bone wax but this is easily remedied because it is water soluble and can be rinsed off.

It is undoubtedly through the mechanism of tamponade that the new material affects immediate hemostasis, but very soon the known specific hemostatic property of oxidized cellulose comes into effect and a plug composed partly of blood, partly of hemostatic bone wax is formed which prevents further bleeding. The exact chemical mechanism bringing about the hemostatic effect of oxidized cellulose has never been completely worked out. In 1944 Frantz, Clarke and Lattes[3] observed that when oxidized gauze is placed in a wound it soon becomes black, swells and forms a sticky, gelatinous mass and bleeding stops quickly. Whatever the nature of this mechanism, numerous experimental and clinical observations on the properties of oxidized cellulose have confirmed this early finding.

After testing a number of samples of the new bone wax containing different amounts of powdered oxidized cellulose and various combinations of polyethylene glycols, the material described in this paper was finally selected because it was thought to have the best physical and hemostatic properties. The formula of the substance is as follows: Carbowax 1540 — 60 per cent; polyethylene glycol 300 — 15 per cent; oxidized cellulose — 25 per cent. However, it is anticipated that further modifications may be made.

CHEMISTRY, TOXICITY AND CLINICAL APPLICATION

There is no need to recapitulate the laboratory and clinical investigations which resulted in establishing oxidized cellulose as a non-toxic, non-irritating absorbable material.[3, 4] The polyethylene glycols are compounds with the general formula: CH_2 —CH_2OH. The lower members of the series are

$$\begin{matrix} CH_2 \\ | \\ O \\ | \\ CH_2 \\ n \end{matrix} \quad -CH_2OH$$

known simply as polyethylene glycols. Those substances with molecular weights of 1000 or higher are solids and are known as carbowax compounds. All these materials are in reality mixtures of a number of closely related polyethylene glycols and are designated by a number which represents the average molecular weight of the various compounds present. Since the only reactive portions of the molecule are the two hydroxyl groups, 83 per cent to 99 per cent of the molecule, depending upon the molecular weight, is chemically inert. Consequently these substances are remarkably stable and enter into few chemical reactions. They are highly soluble in water. An extensive series of experiments to determine their toxicity was undertaken by Smythe and his collaborators in 1941.[17, 18] These workers have shown that in contrast to the first two members of the group, diethylene and triethylene glycol, which have a low molecular weight and are liquids, the toxicity of the higher members is extremely low. A considerable amount of data on the toxicology of these

TABLE I.—*Absorbable Bone Wax in Rats.*

Number of Animals	Length of Time in Tissues	Gross Appearance		
		Residual Material	Tissue Fluid at Implantation Site	Blood Vessel Engorgement
3	1 hour	Partly liquefied	None	Slight
4	2 hours	Liquid remnant or no residual	Slight or none	Slight or none
3	3 hours	None	Slight or none	Slight
3	4 hours	None	Slight or none	Moderate
9	24 hours	None	No fluid in four; 1 — 3 cc. in five	Slight to none in four; moderate in five with fluid
4	4 days	None	2 cc. fluid in one; no fluid in three	Slight to moderate
9	7 days	None	None	None to moderate
4	10 days	None	None	Slight to none

compounds was accumulated in experiments which included oral, intraperitoneal and topical administration to a number of different kinds of laboratory animals over varying periods of time up to two years. Application to the skin of human beings showed that these substances are no more irritating than a number of other commonly employed bases, such as cocoa butter, glycerol and lanolin. These investigations led to the clinical use of the compounds for topical application. Experimental and clinical confirmatory studies were made by Dodd, Hartmann and Ward[2]; Friedman[5]; Shaffer and Critchfield[15]; Shaffer, Critchfield and Carpenter[16]; Reid and Altemeier[13]; Cochran[1]; Meleney, Johnson, Pulaski and Colonna[11]; Hopkins[7]; and Maynard.[10]

EXPERIMENTAL OBSERVATIONS

Our experimental studies of the new absorbable hemostatic bone wax were undertaken in two phases. In one group of experiments we studied the rate of absorption and irritating effects of the material by means of the standard Lattes-Frantz rat test[9] which has been used extensively in this laboratory in the testing of tissue reactions to foreign materials. Concurrently, experiments

were initiated to determine the effect of the new material on the healing of experimental rib fractures in dogs, comparing these results with the healing observed when ordinary bone wax or oxidized cellulose alone was implanted at the fracture site.

1. Rat Tests. The results of these experiments are summarized in Tables I and II. On gross examination the new bone wax was completely absorbed in all animals at the end of three hours. Microscopic examination at this time, however, revealed particles of oxidized cellulose. At 24 hours some tissue fluid was present at the implantation site in five of the nine animals, but at four days fluid was present in only one out of four. Moderate congestion of the neighboring blood vessels was seen in half the animals (Figs. 1 and 2).

TABLE II.—*Ordinary Bone Wax in Rats.*

Number of Animals	Length of Time in Tissues	Gross Appearance		
		Residual Material	Tissue Fluid at Implantation Site	Blood Vessel Engorgement
1	4 hours	No change	None	None
1	24 hours	No change	None	Slight
4	7 days	Three with cysts averaging approximately 1.5 cm.; one with mass of wax measuring 1.0 cm. in diameter.		Severe

Histologic examination during the first four hours revealed the persistence of oxidized cellulose particles and a moderate number of inflammatory cells. One interesting and unexplained observation was the predominance of eosinophilic leukocytes among these cells. The remainder of the inflammatory infiltrate was made up of polymorphonuclear leukocytes and mononuclear phagocytes. The reason for this early eosinophilic infiltration is not clear because the distribution of leukocytes in the peripheral blood of the rat resembles that of man quite closely.[14] This phenomenon was not observed at 24 hours or later.

The maximum inflammatory reaction was observed at 24 hours, when a moderately intense infiltration of cells was present. About half the cells were polymorphonuclear leukocytes and the remainder were lymphocytes, plasma cells and mononuclear phagocytes. Numerous irregular, basophilic masses were observed which were thought to represent oxidized cellulose particles which had partly dissolved and coalesced. At four days only young granulation tissue with many capillaries was seen, and at seven and ten days denser, more mature fibrous tissues was observed (Figs. 3 and 4).

In the series of control animals in which ordinary bone wax was used, no significant change in the implant was noted in the first 24 hours. At seven days all showed an intense inflammatory reaction, three having cysts containing sterile fluid and numerous particles of wax and a fourth presenting a single, large encapsulated mass of wax. Histologic examination revealed numerous polymorphonuclear leukocytes and an occasional foreign body giant cell (Figs. 5 and 6).

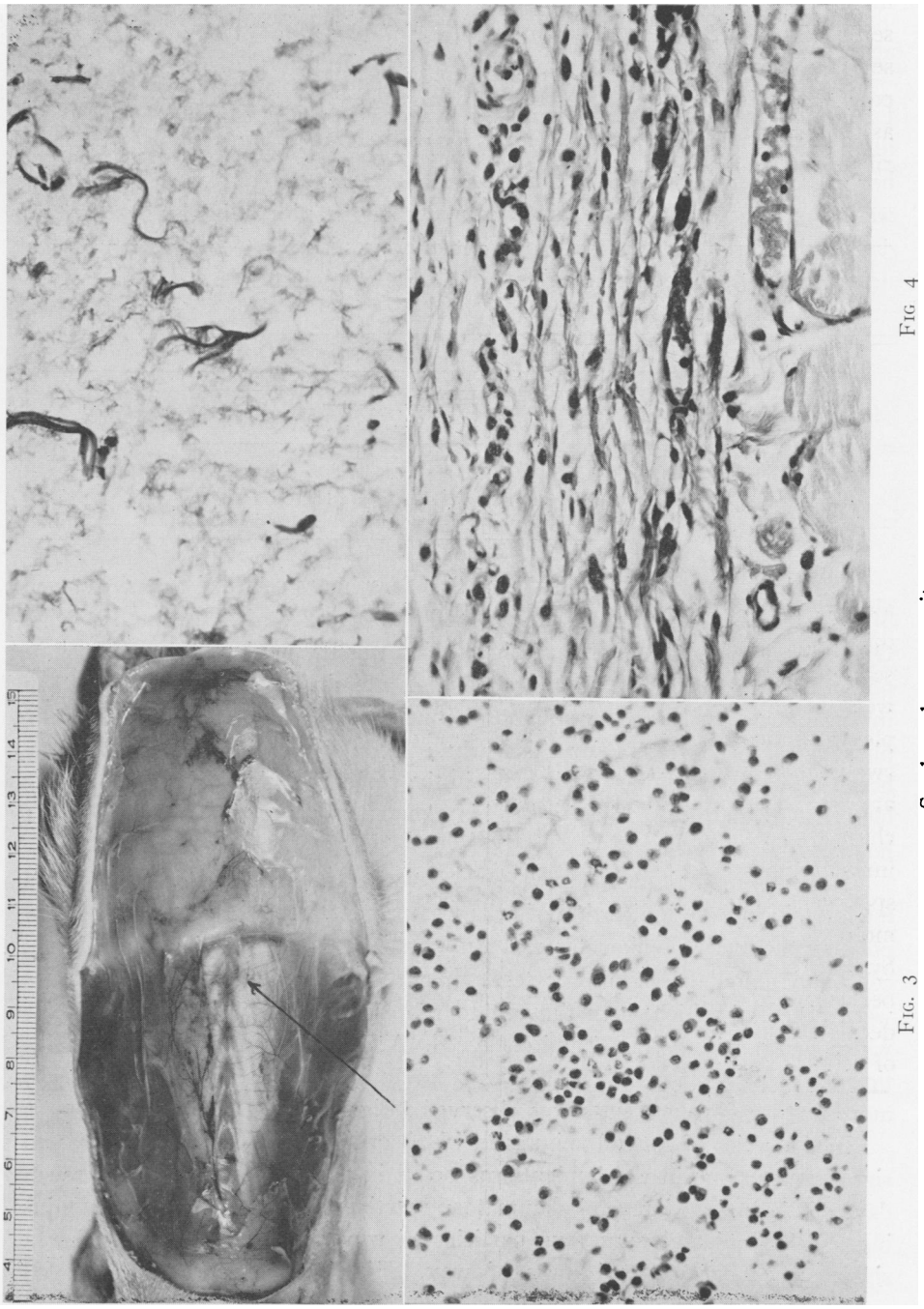

FIG. 1

FIG. 2

FIG. 3

FIG. 4

See legends on opposite page.

2. Rib Implants. The results of the rib experiments in dogs are summarized in Table III. The sixth, seventh, eighth and ninth ribs were used in the first series of animals with implants in order as follows: sixth rib—new bone wax; seventh rib—ordinary bone wax; eighth rib—oxidized cellulose; ninth rib—control. In the second series the sixth, eighth and tenth ribs were employed as follows: sixth rib—new bone wax; eighth rib—ordinary bone wax; tenth rib—control.

TABLE III.—*Experimental Rib Fractures in Dogs*

		X-Ray Evidence of Repair				Microscopic Evidence of Bone Formation			
Animal	Number of Days	New BoneWax	Ordinary BoneWax	Oxidized Gauze	Control	New BoneWax	Ordinary BoneWax	Oxidized Gauze	Control
A	2	0	0	..	0	0	0	..	0
B	9	0	0	0	0	+	+	+	+
C	14	0	0	..	0	2+	2+	..	2+
D	23	2+	3+	+	3+	3+	3+	3+	3+
E	30	+	+	..	+	2+	+	..	2+
F	59	union	union	union	union	union	union	union	union
G	91	3+	3+	2+	union	3+	3+	2+	union
H	120	union	union	union	union	union	union	union	union

At the time of necropsy, all wounds appeared to be healing well or had already completely healed. In two cases, autopsied at 59 and 91 days after operation respectively, a number of small particles of ordinary bone wax were scattered widely in the lower portion of the wounds. The pleural surfaces were in all cases intact and appeared pink and glistening. There were no pleural adhesions. The sites of the fractures appeared as smooth, fusiform swellings in the course of the ribs. The degree of immobility already achieved at each fracture site was carefully estimated and recorded. The portion of chest wall containing the fractures was then removed in one segment and immediately placed in a deep-freezing unit. Roentgenograms were taken of all specimens after they had been frozen. The ribs were then sectioned on a small, motor-driven jigsaw and decalcified during a period of from seven to ten days by daily changes of a freshly made up solution consisting of equal parts of 50 per cent formic acid and 20 per cent sodium citrate. Complete and satisfactory decalcification was accomplished by this method with fairly good preservation of cellular details.

FIG. 1.—Absorbable bone wax 24 hours in subcutaneous tissues of back of rat. Complete absorption of material and moderate congestion of blood vessels. (Arrow indicates site of implant.)

FIG. 2.—Absorbable bone wax three hours in back of rat. High power photomicrograph shows fibers of oxidized cellulose and a few inflammatory cells on a background of fibrin strands.

FIG. 3.—Absorbable bone wax 24 hours in subcutaneous tissues of back of rat. Moderately dense infiltration of inflammatory cells consisting of polymorphonuclear leukocytes, plasma cells, lymphocytes and large, mononuclear phagocytes. No residual material.

FIG. 4.—Absorbable bone wax seven days in subcutaneous tissues of back of rat. High power photomicrograph shows cellular connective tissue containing several capillaries.

Active proliferation of new bone from the cambium layer of the periosteum and from the endosteum was first observed in the nine-day fractures. This was noted at all fracture sites at this time. The first definite evidence of calcium deposition, as determined by roentgen rays, occurred at 23 days and bony union at all fracture sites first occurred at 59 days.

Although some difference could be detected histologically in the rate of healing at the different fracture sites, these were not nearly as striking as the

FIG. 5

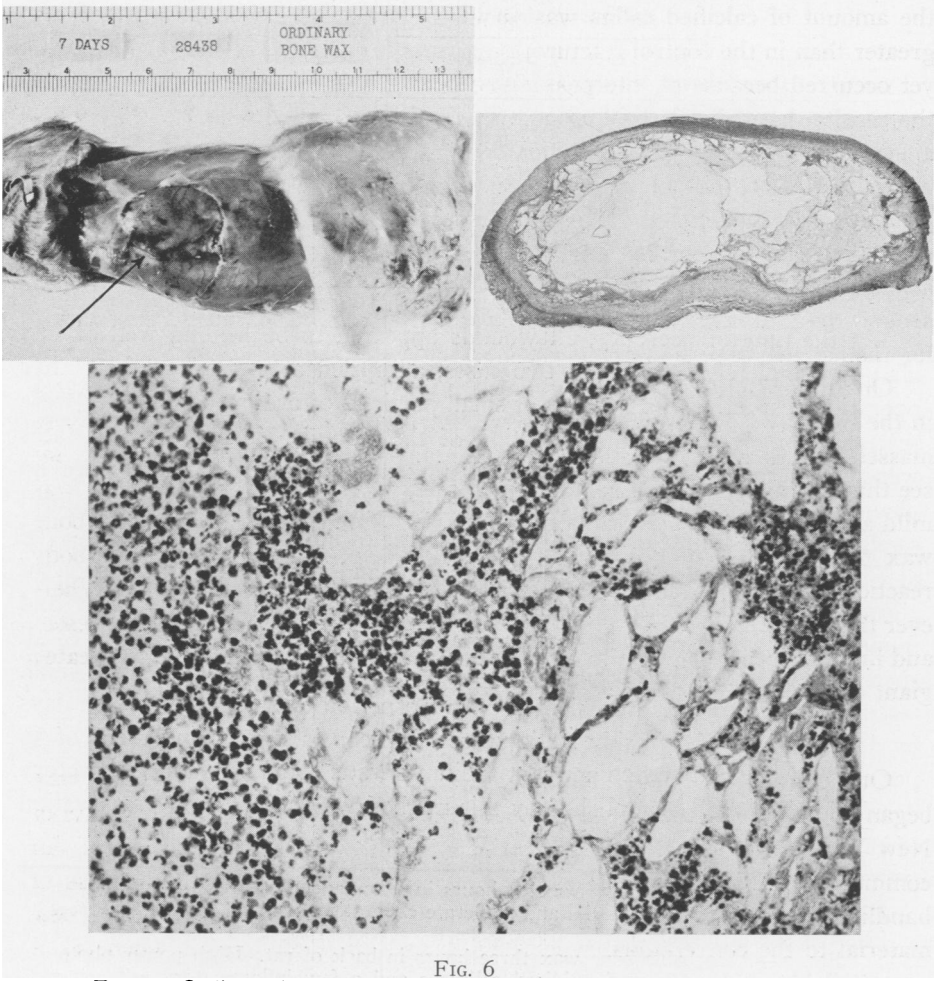

FIG. 6

FIG. 5.—Ordinary bone wax seven days in subcutaneous tissues of back of rat. Upper photograph shows 1.0 x 1.8 x 2.5 cm. cyst (indicated by arrow) which contained thin, brown fluid and particles of wax. Culture negative. Below is low power photomicrograph of cross-section of cyst. See Figure 6 for high power photomicrograph of wall of cyst.

FIG. 6.—Ordinary bone wax seven days in subcutaneous tissues of back of rat. High power photomicrograph of section of cyst shown in Figure 5. Numerous polymorphonuclear leukocytes and irregular, clear spaces which were formerly occupied by particles of wax.

differences in the degree of calcification as demonstrated by the roentgen rays. In some instances there was little or no difference in the histologic appearance of two fractures but a marked and striking difference would be noted in the roentgenogram. This suggested to us that the observed differences were not due so much to differences in the proliferation of osteoid tissue but were rather due to differences in the rate of calcification. In general the roentgen rays showed that the amount of calcified callus present at the site where the new absorbable bone wax was used was either about the same or somewhat less than the amount at the control fracture. Where ordinary bone wax was used the amount of calcified callus was about the same or occasionally somewhat greater than in the control fracture, even though union of the fracture had not yet occurred because of interposition of particles of wax. It is evident from the results that delay in healing occurs with both types of wax but the mechanism appears to be different in the two cases. With ordinary bone wax the delay is probably a result of mechanical factors. The particles of wax remain between the fragments and thus prevent union. With absorbable wax, however, the delay is probably due to alterations in the pH of the tissues. The acidity of the oxidized cellulose undoubtedly lowers the pH of the tissues around the fracture site and thus delays the "alkaline tide" which is essential for the activity of alkaline phosphatase and the deposition of calcium.[12]

On microscopic examination, we observed some irregular basophilic masses in the two-day fracture where the new absorbable bone wax was used. These masses were probably fragments of residual oxidized cellulose. We did not see this material in any of the later fractures. The inflammatory reaction was mild and had completely disappeared after the second day. The ordinary bone wax produced a very characteristic histologic appearance and foreign body reaction, which was easily recognizable as late as the fifty-ninth day. Wherever the wax remained in the tissues, many irregular, clear spaces were present and in these areas would be found numerous, irrgularly-shaped, multinucleated giant cells; lymphocytes; plasma cells and mononuclear phagocytes.

CLINICAL OBSERVATIONS

Our clinical experience with the new absorbable hemostatic bone wax began on March 15, 1948, when the surgeons at the Neurological Institute of New York began the routine use of this material in the operating rooms. All comments by these surgeons regarding its hemostatic properties and ease of handling have been entirely favorable. The surgeons have preferred the new material to the conventional type of bone wax and have used it whenever it was available.

The first 100 consecutive operations in which the new material was employed are tabulated in Table IV. The wounds in 98 of this group developed no infections and healed per primam. In one case, (U. H. 919513) following a laminectomy, a small, superficial abscess developed around a black silk suture. The wound healed promptly after the removal of the suture. In the

other case (U. H. 919223) a large, left fronto-parietal brain abscess was evacuated and the abscess cavity lined with China silk and packed with gauze. This wound continued to drain for a number of weeks.

Out of this group of 100 operations, it was possible to detect significant amounts of fluid under the scalp or in the wound in seven cases. In two cases the fluid was absorbed spontaneously and did not require tapping. Two cases were tapped only once (U. H. 904703) following a bilateral topectomy and (U. H. 890677) following a craniotomy and lysis of Pacchionian granulations. The remaining three patients required repeated taps. One patient (U. H. 725805) had a suboccipital craniectomy with the subtotal removal of a cystic

TABLE IV.—*Clinical Experience With New Absorbable Bone Wax**

Differential section of trigeminal nerve	11
Pre-frontal leukotomy	7
Trephination of skull	5
Craniotomy	39
Sub-occipital craniectomy	4
Topectomy	6
Laminectomy	16
Interlaminar removal of herniated nucleus pulposus	12
Total	100

*Since this article was submitted for publication the new bone wax has been used in a considerable number of general surgical cases. Limited quantity has delayed general trial, but more material will be available as soon as the optimum consistency has been determined.

astrocytoma of the cerebellum. The other two patients (U. H. 897548) and (U. H. 910605) each had the removal of a large meningioma which was then followed by a tantalum cranioplasty. We do not feel that the new absorbable bone wax caused the production of fluid in any of these cases, but believe, rather, that the fluid was a result of the operative procedure itself. In support of this view is the observation that in a number of other cases as many as three tubes of the material, each tube containing three grams of absorbable bone wax, were used following which the wound healed per primam and no fluid collected. Further records were kept but have not been analyzed as the wax has been used routinely.

SUMMARY

A new absorbable hemostatic bone wax has been produced which contains oxidized cellulose as its active component and a mixture of polyethylene glycols of high molecular weight as the vehicle. This material has been found to be actively hemostatic when employed to control hemorrhage from the bleeding surfaces of bone and can be handled and applied more easily than ordinary bone wax.

Laboratory tests with rats indicated that the new material is much less irritating to the tissues than ordinary bone wax and is completely absorbed in three hours.

In experimental rib fractures in dogs both the new material and ordinary bone wax caused some delay in healing. It is not felt that this delay is of any practical significance, however, since the material will not be used in accidental fractures, where early bone repair is the first consideration, nor in weight-bearing bones. In any event, the delay does not seem to be greater than that caused by ordinary bone wax which has been used for the same purpose for over 50 years.

The use of new absorbable hemostatic bone wax in the first 100 clinical neurosurgical operations at the Neurological Institute of New York has been reported. No ill effects or complications which could be attributed to the material have been noted.

Note: The authors wish to express their appreciation to Miss Daisy Mapes, R. N. under whose supervision the animal experiments were carried out.

BIBLIOGRAPHY

[1] Cochran, J. R.: A New Ointment Containing Zinc Peroxide. Quart. Bull., Northwestern Univ. M. School, **18**: 41, 1944.

[2] Dodd, M. C., F. W. Hartmann and W. C. Ward: Development of a Water-Soluble Vehicle (Base C-7) for Furacin. (Privately published and distributed by the Eaton Laboratories, Inc., Norwich, N. Y.)

[3] Frantz, V. K., H. T. Clarke and R. Lattes: Hemostasis with Absorbable Gauze (Oxidized Cellulose). Ann. Surg., **120**: 181, August, 1944.

[4] Frantz, V. K., and R. Lattes: Oxidized Cellulose—Absorbable Gauze (Cellulosic Acid). J. A. M. A., **129**: 798, 1945.

[5] Friedman, M. H. F.: Vehicle for Intravenous Administration of Fat-Soluble Hormones. J. Lab. & Clin. Med., **29**: 530, 1944.

[6] Henderson, J.: Personal communication.

[7] Hopkins, J. G.: Some Newer Bases for Use in Cutaneous Therapy. J. Invest. Dermat., **7**: 171, 1946.

[8] Horsley, V.: Antiseptic Wax. Brit. M. J., **1**: 1165, 1892.

[9] Lattes, R., and V. K. Frantz: Absorbable Sponge Tests. Ann. Surg., **121**: 894, 1945.

[10] Maynard, M. T. R.: Polyethylene Glycol Vehicles in Dermatology. J. Invest. Dermat., **8**: 223, 1947.

[11] Meleney, F. L., B. Johnson, E. J. Pulaski and F. Colonna: Treatment of Mixed Infections with Penicillin. J. A. M. A., **130**: 121, 1946.

[12] Murray, C. R.: The Timing of the Fracture-Healing Process. J. Bone & Joint Surg., **23**: 598, 1941.

[13] Reid, M. R., and W. A. Altemeier: Peroxide Ointments. Ann. Surg., **118**: 741, 1943.

[14] Scarborough, R. A.: The Blood Picture of Normal Laboratory Animals. The Rat. Yale J. Biol. & Med., **2**: 267, 1931.

[15] Shaffer, C. B., and F. H. Critchfield: The Absorption and Excretion of the Solid Polyethylene Glycols; (Carbowax Compounds). J. Am. Pharm. (Scient. Ed.), **36**: 152, 1947.

[16] Shaffer, C. B., F. H. Critchfield and C. P. Carpenter: Renal Excretion and Volume Distribution of Some Polyethylene Glycols in the Dog. Am. J. Physiol., **152**: 93, 1948.

[17] Smyth, H. F., C. P. Carpenter and C. B. Shaffer: Subacute Toxicity and Irritation of Polyethylene Glycols of Approximate Molecular Weights of 200, 300 and 400. J. Am. Pharm. A., (Scient. Ed.), **34**: 172, 1945.

[18] Smyth, H. F., C. B. Shaffer and C. P. Carpenter: The Toxicity of High Molecular Weight Polyethylene Glycols; Chronic Oral and Parenteral Administration. J. Am Pharm. A., (Scient. Ed.), **36**: 157, 1947.