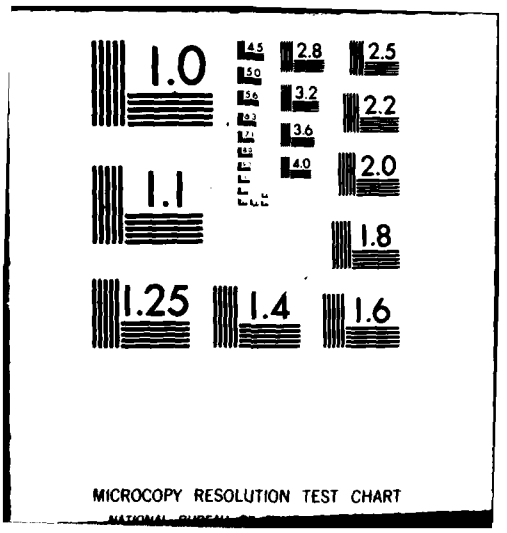
AD-A091 901 ARMY INST OF DENTAL RESEARCH WASHINGTON DC F/G 6/15
A COMPARISON OF THE INFLAMMATORY RESPONSE PRODUCED BY COMMERCIA--ETC(U)
MAY 80 J G WEBB, N E BUSSELL

UNCLASSIFIED NL

END
DATE FILMED
1-81
DTIC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
MICROCOPY RESOLUTION TEST CHART

LEVEL II

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO. AD-A091901

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
A Comparison of the Inflammatory Response Produced by Commercial Eugenol and Purified Eugenol

5. TYPE OF REPORT & PERIOD COVERED
Manuscript for Publication

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Joseph G. Webb, Jr. and Norman E. Bussell

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U. S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, DC 20012

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
Program Element: 61102A, Project: 3S161102BS06, Task Area 04, Work Unit #010

11. CONTROLLING OFFICE NAME AND ADDRESS
U. S. Army Medical Research & Development Command
HQDA-IS
Fort Detrick, Maryland 21701

12. REPORT DATE
22 May 1980

13. NUMBER OF PAGES
17

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
This document has been approved for public release and sale; its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

DTIC ELECTE NOV 7 1980 S C

18. SUPPLEMENTARY NOTES
None

Supersedes AD-A086402 (Incomplete)

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Eugenol is one of the major therapeutic agents in dentistry. The inflammatory response to purified and commercial eugenol are compared, and the inflammatory response to zinc oxide/eugenol prepared with either purified or commercial eugenol are also compared. The results indicate that eugenol is inflammatory but the impurities present in commercial eugenol increase the inflammatory response to eugenol and zinc oxide eugenol.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE UNCLASSIFIED

AD A091901

DDC FILE COPY

11 05 021

A COMPARISON OF THE INFLAMMATORY RESPONSE PRODUCED BY COMMERCIAL EUGENOL AND PURIFIED EUGENOL

Joseph G. Webb, Jr.*
Norman E. Bussell†

*Chief, Oral Pathology
U. S. Army DENTAC
Fort Ord, California

†Division of Oral Biology
U. S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D. C.

SYNOPSIS

Eugenol is one of the major therapeutic agents in dentistry. The inflammatory response to purified and commercial eugenol are compared, and the inflammatory response to zinc oxide/eugenol prepared with either purified or commercial eugenol are also compared. The results indicate that eugenol is inflammatory, but the impurities present in commercial eugenol increase the inflammatory response to eugenol and zinc oxide eugenol.

INTRODUCTION

Zinc oxide/eugenol (ZOE) preparations have been used as sedative preparations, as anodyne dressings, as pulp capping agents, and in root canal materials. The dental literature contains many articles concerning the inflammatory response to ZOE[1-19] and most authors agree that ZOE is an irritant. However, commercial or "stock" eugenol contains a number of impurities;[20,21] e.g. acetyl eugenol, furfural, methyl-n-amyl-ketone, and others, many of which may contribute to the inflammatory reaction. It should be noted that considerable variation in the impurities exists between batches, as previously shown.[20] Molnar,[22] in a study of residual eugenol from ZOE compounds, suggested that the variations in periapical reactions to ZOE mixtures may be a direct result of the residual (free) eugenol remaining after the compound has set. To date, the degree of inflammation created specifically by the eugenol component of a ZOE preparation has not been adequately determined.

Our laboratory has purified eugenol to greater than 99.75% purity by HPLC technique.[20] This investigation was undertaken to evaluate the inflammatory response of soft tissue to commercial eugenol and to a purified eugenol. We also sought to determine if the impurities in a commercial eugenol increase its inflammatory response.

MATERIALS AND METHODS

Forty adult male Walter Reed white rats, each weighing 250-300 grams, were used for this study. Each rat was placed in a restraining device and its belly was shaved. Each material was injected subcutaneously into each animal :0.1 cc of commercial USP eugenol,* 0.1 cc of purified eugenol

*Gentry International, Fairlawn, New Jersey
(Lot #B14997) FSN 6505-00-153-8379

(prepared as previously reported[20]), 0.1 cc of commercial eugenol combined with zinc oxide, and 0.1 cc of purified eugenol combined with zinc oxide. The zinc oxide eugenol mixtures were prepared using 0.40 gm zinc oxide and 0.10 cc of eugenol. All animals were injected with the ZOE within five minutes after mixing. To compensate for any possible local variation in injection sites, each material was used an equal number of times on the right and left of the midline of the belly of each rat.

The 40 animals were then divided into four groups of ten animals each. The trauma of injecting the materials into the rat produced some inflammatory response, so the first group of ten animals was maintained for two days before sacrificing. Each experimental animal was sacrificed by intracardiac injection of 10% buffered formalin. The remaining three groups of animals were sacrificed on day 6, day 10, and day 15. The skin and some subcutaneous tissue was removed from each injection site and placed in 10% formalin. After being imbedded in paraffin, serial sections were cut and stained with hemotoxylin and eosin for histologic examination.

ANALYSIS OF DATA

The degree of inflammation and necrosis was subjectively graded at each injection site by three independent pathologists, using a scale of 1 to 4, with 1 indicating minimal inflammation and 4 indicating severe inflammation. The data for the degree of inflammation was compared using mean values, (Table I) and the degree of necrosis was evaluated using the same technique (Table II). The results are also shown in Figures 1 and 2.

RESULTS

2 Day: A large area of necrosis at the injection sites was present in all animals injected with both the purified and the commercial eugenol. The injection of the purified ZOE and stock ZOE also produced some necrosis

in the tissue immediately adjacent to the material, but the area of necrosis was much less than that produced by the injection of either eugenol alone (Figure 3A). The smallest amount of necrosis was observed with the pure ZOE. The commercial and purified ZOE specimens contained a larger number of inflammatory cells (polymorphonuclear leukocytes, plasma cells and lymphocytes) in the immediate vicinity of the material than the purified and commercial eugenol specimens.

6 Day: The necrotic areas produced by the purified eugenol and commercial eugenol were smaller after six days. The purified ZOE and commercial ZOE also had a slightly reduced area of necrosis, with the purified ZOE again showing the smallest areas of necrosis (Figure 3B). The number of inflammatory cells increased in all specimens, with the purified and commercial ZOE again having the largest number.

10 Day: The amount of necrosis with the purified eugenol decreased significantly from the 6 day specimens. The commercial eugenol was relatively unchanged from day 6. There was an increase in the amount of necrosis seen around both the purified and commercial ZOE mixtures. The number of inflammatory cells was greater in all of the 10 day specimens than in any of the previous groups (Figure 3C).

15 Day: The necrotic material in the injection sites was greatly reduced for all groups except the commercial ZOE. This group had less necrosis than in the 10 day sample, but a significant difference existed between this commercial ZOE and the other three groups (Figure 3D). The inflammatory infiltrate had likewise greatly decreased for all specimens except the commercial ZOE, which still contained a large number of chronic inflammatory cells.

DISCUSSION

With both the purified and commercial eugenols alone, an area of necrosis was evident at the injection site within the first 24 hours. The amount of necrosis slowly subsided for both liquids. The number of inflammatory cells reached a peak at 10 days, with the stock eugenol eliciting a greater inflammatory response at all dates. At 15 days, the difference in inflammatory response between the purified and commercial eugenols was the greatest. Thus, when compared to commercial eugenol, the purified eugenol elicits less of an inflammatory response at day 2, a similar response at day 6 and day 10, and considerably less of an inflammatory response at day 15.

Injection of mixtures of each of the two eugenols with zinc oxide resulted in less initial necrosis, with a larger degree of necrosis at day 6 and day 10. By day 15, the amount of necrosis had greatly decreased, especially for the purified ZOE. The amount of necrosis with the commercial ZOE was significantly greater than that occurring with the purified ZOE.

The inflammatory response elicited by both mixtures of ZOE increased from day 2 through day 6, to peak at day 10. There was only a small difference between the mixtures at these three dates, but the purified ZOE consistently had fewer inflammatory cells present. At day 15 the amount of inflammation was reduced for the commercial ZOE, and greatly reduced for the purified ZOE.

Our findings suggest that the purified eugenol produced slightly less necrosis at the sacrifice dates than that produced by the stock eugenol. The inflammatory response was consistently lower with the

purified eugenol, with the largest differences initially (day 2) and at day 15. This indicates that commercial eugenol produces greater inflammation initially, and the inflammation remains more severe for a longer period of time.

The commercial ZOE consistently produced more necrosis than the purified ZOE at all dates of sacrifice, with both materials causing the most tissue destruction at day 10. The number of inflammatory cells was also greatest for both ZOE materials at day 10, with the largest difference in inflammation evident at day 15. Thus, the commercial ZOE produces more necrosis and inflammation at all dates than does the purified ZOE, with the greatest differences evident after day 10.

Figures 1 and 2 show the relationship between time and the degree of necrosis and inflammation, respectively. These figures demonstrate that the purified and commercial eugenols roughly parallel each other, with the commercial eugenol causing more necrosis and inflammation at all dates. The figures for the purified and commercial ZOE mixtures also are roughly parallel, with the commercial eugenol causing more necrosis and inflammation at each date. Figure 2 also shows that the two mixtures of ZOE roughly parallel the two eugenol lines, with all four materials causing the most inflammation at day 10. This lends some support to the theory[21] that the inflammatory response produced by ZOE mixtures is strongly influenced by the amount of free eugenol.

SUMMARY AND CONCLUSIONS

Eugenol "purified" by HPLC[20] was compared to commercial USP eugenol to determine if any difference exists between the inflammatory response

caused caused by each. A mixture of each eugenol with zinc oxide were also compared. Each material was injected subcutaneously in the belly of forty Walter Reed white rats. Ten animals were sacrificed at four different dates and the degree of necrosis and inflammation was compared histologically. The purified eugenol caused less necrosis and inflammation at all dates than the commercial eugenol. The purified ZOE mixture produced less necrosis and inflammation than the commercial ZOE mixture at each sacrifice date. The two mixtures of ZOE and the two samples of eugenol produced roughly parallel amounts of inflammation when graphed, suggesting that the degree of inflammation of ZOE mixtures is strongly influenced by the amount of free eugenol in the mixtures. This study suggests that the impurities in commercial eugenol do cause an increase in the inflammatory response, and this increase is most evident at day 2 and after day 10.

In conducting the research described in this study, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

* * * * * * *

The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense. Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose.

TABLE I. Mean Values of Inflammation* ± 1 S.D.

Group	Number of Animals	2 Day	6 Day	10 Day	15 Day
Purified Eugenol	40	1.4±0.5	2.2±0.4	2.8±0.4	1.6±0.8
Commercial Eugenol	40	2.2±0.4	2.3±0.4	3.0±0.6	2.6±0.5
Purified ZOE	40	2.2±0.4	3.4±0.8	3.8±0.4	2.8±0.7
Commercial ZOE	40	2.2±0.4	3.6±0.5	4.0±0.0	3.4±0.5

* 1 = Minimal Inflammation, 4 = Maximum Inflammation

TABLE II. Mean Values of Necrosis* ± 1 S.D.

Group	Number of Animals	2 Day	6 Day	10 Day	15 Day
Purified Eugenol	40	4.0±0.0	2.6±0.6	2.0±0.6	1.1±1.0
Commercial Eugenol	40	4.0±0.0	2.8±0.7	2.6±0.5	1.4±0.5
Purified ZOE	40	2.0±0.0	1.4±0.5	2.2±0.4	1.0±0.0
Commercial ZOE	40	2.6±0.5	2.0±0.0	3.0±0.0	2.2±0.5

* 1 = Minimal Necrosis, 4 = Maximum Necrosis

LEGENDS

Figure 1. Degree of Necrosis vs. Time

Figure 2. Degree of Inflammation vs. Time

Figure 3A. Two-Day Specimen of Purified ZOE Mixture Showing Areas of Necrosis and the Inflammatory Response. 40X

Figure 3B. Purified ZOE at Day 6. 40X

Figure 3C. Purified ZOE at Day 10 Showing an Increased Number on Inflammatory Cells. 100X

Figure 3D. Day 15 Specimen of Commercial ZOE. 100X

REFERENCES

1. GURLEY, W. B. and VAN HUYSEN, G.: Histologic Changes in Teeth Due to Plastic Filling Materials, J Am Dent Assoc 24:1806-1816, 1937.
2. JAMES, V. E. and DIEFENBACH, G.B.: Prevention of Histological Changes in Young Dog's Teeth by Use of Zinc Oxide and Eugenol. J Am Dent Assoc 29:583-592, 1942.
3. JAMES, V. E. and SCHOUR, I.: Early Dentinal and Pulpal Changes Following Cavity Preparations and Milling Materials in Dogs. Oral Surg 8:1305-1314, 1955.
4. SILBERKWEIT, M.; MASSLER, M.; SCHOUR, I.; and WEINMANN, J.: Effects of Filling Materials on the Pulp of the Rat Incisor. J Dent Res 34:854-869, 1955.
5. MITCHELL, D. R.: The Irrational Qualities of Dental Materials. J Am Dent Assoc 59:954-966, 1959.
6. ROBINSON, H.B.G. and LEFKOWITZ, W.: Operative Dentistry and the Pulp. J Prosthet Dent 12:985-1001, 1962.
7. DUBNER, R. and STANLEY, J.: Reaction of the Human Dental Pulp to Temporary Filling Materials. Oral Surg 15:1009-1017, 1962.
8. STANLEY, H.R.; SWERDLOW, H; and BUONOCORE, M.G.: Pulp Reaction to Anterior Restorative Materials. J Am Dent Assoc 75:132-144, 1967.
9. BHASKAR, S.; CUTRIGHT, D.E.; BEASLEY, J.; and BOYERS, R.: Pulpal Response to Four Restorative Materials. Oral Surg 28:126-133, 1969.
10. GOTO, G. and JORDAN, R.E.: Pulpal Response to Composite Resin Material. J Prosthet Dent 28:601-606, 1972.

11. AVERY, J.: Response to Pulp and Dentin to Contact with Filling Materials. J Dent Res 54:B188-B197, 1975.

12. STANLEY, H.R.; GOING, R.E.; and CHANCERY, H.H.: Human Pulp Response to Acid Pretreatment of Dentin and Composite Restoration. J Am Dent Assoc 91:817-825, 1975.

13. WEISS, M.B. and BJORVATN, K.: Pulp Capping in Deciduous and Newly Erupted Permanent Teeth of Monkeys. Oral Surg 29:769-775, 1970.

14. OBERSZTYN, A.: Healing of Pin Exposure of Rat Incisor Pulp Under Various Capping Agents. J Dent Res 45:1130-1142, 1966.

15. SELA, J. and ULMANSKY, M.: Reaction of Normal and Inflamed Dental Pulp to Calxyl and Zinc Oxide and Eugenol in Rats. Oral Surg 30:425-430, 1970.

16. NIXON, G.S. and HANNAH, C. McD.: N-butyl Cyanoacrylate as a Pulp Capping Agent. Br Dent J 133:14-18, 1972.

17. CURSON, I. and KIRK, E.E.J.: An Assessment of Root Canal Sealing Cements. Oral Surg 26:229-236, 1968.

18. RAPPAPORT, H.; LILLY, G.; KAPAIMALIS, B.: Toxicity of Endodontic Filling Material. Oral Surg 18:785-802, 1964.

19. ERAUSQUIN, J. and MURUZABAL, M.: Tissue Reaction to Root Canal Cements in the Rat Molar. Oral Surg 26:360-373, 1968.

20. MILLER, R.A.; BUSSELL, N.E.; RICKETTS, C.K.; and JORDI, H.: Analysis and Purification of Eugenol. J Dent Res 58:1394-1400, 1979.

21. NTAMILA, M.S. and HASSANALI, A.: Isolation of Oil of Clove and Separation of Eugenol and Acetyl Eugenol. J Chem Educ 53:263, 1976.

22. MOLNAR, E.J.: Residual Eugenol from Zinc Oxide Eugenol Compounds. J Dent Res 46:645-649, 1967.

Requests for Reprints to:

Norman E. Bussell, MAJ, DC
Division of Oral Biology
U. S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D. C. 20012

NECROSIS vs TIME

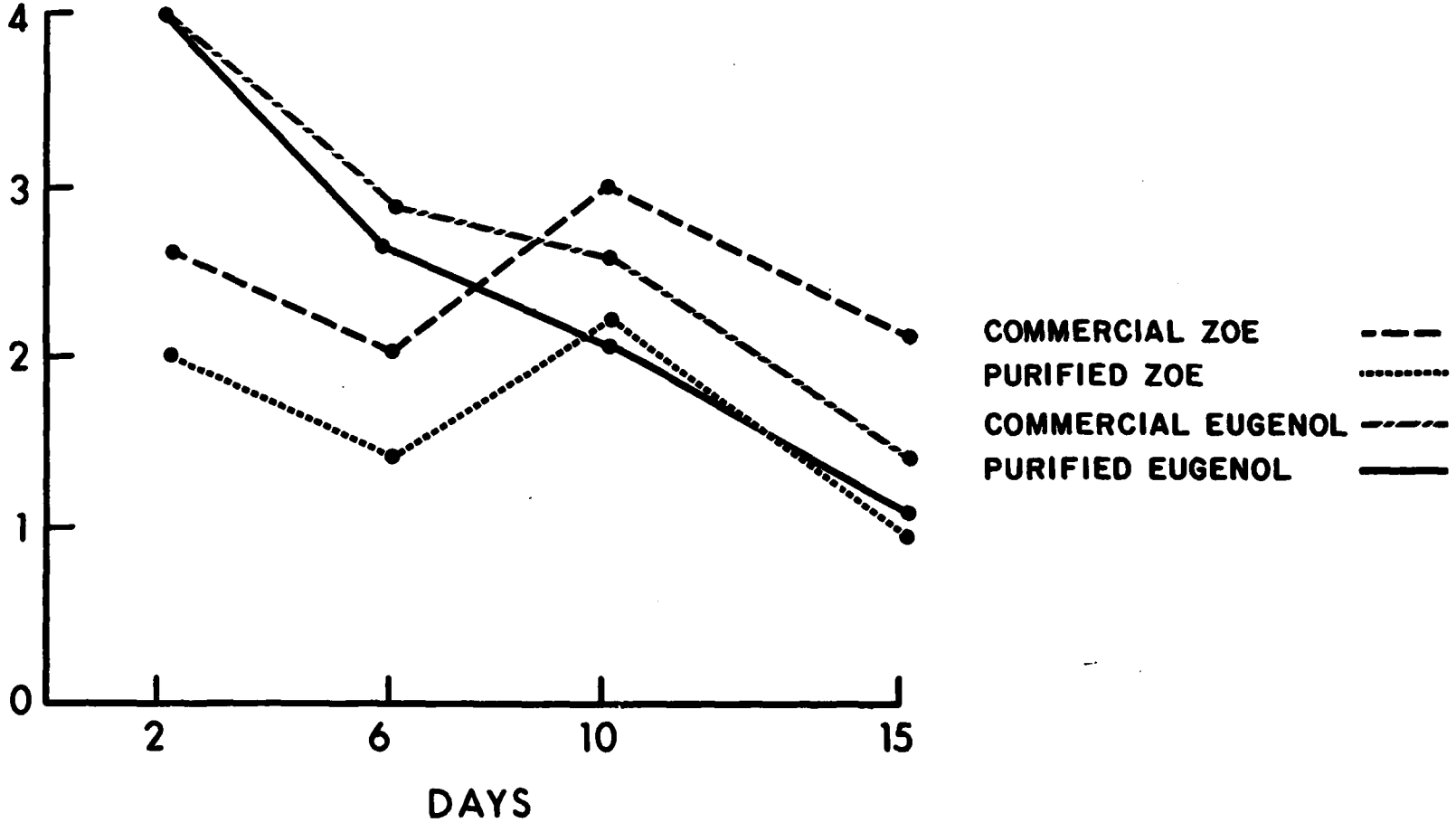


Fig 1

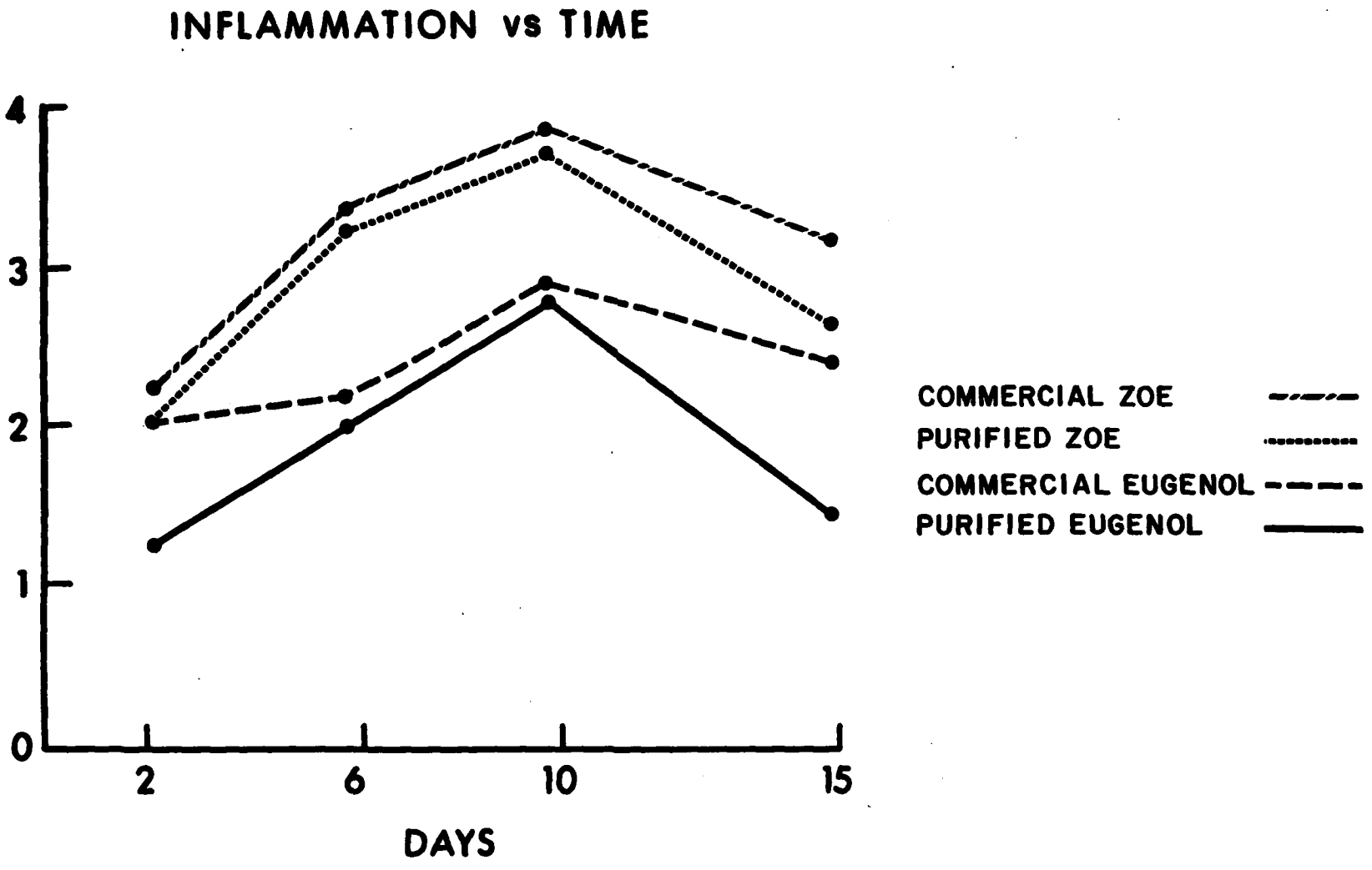
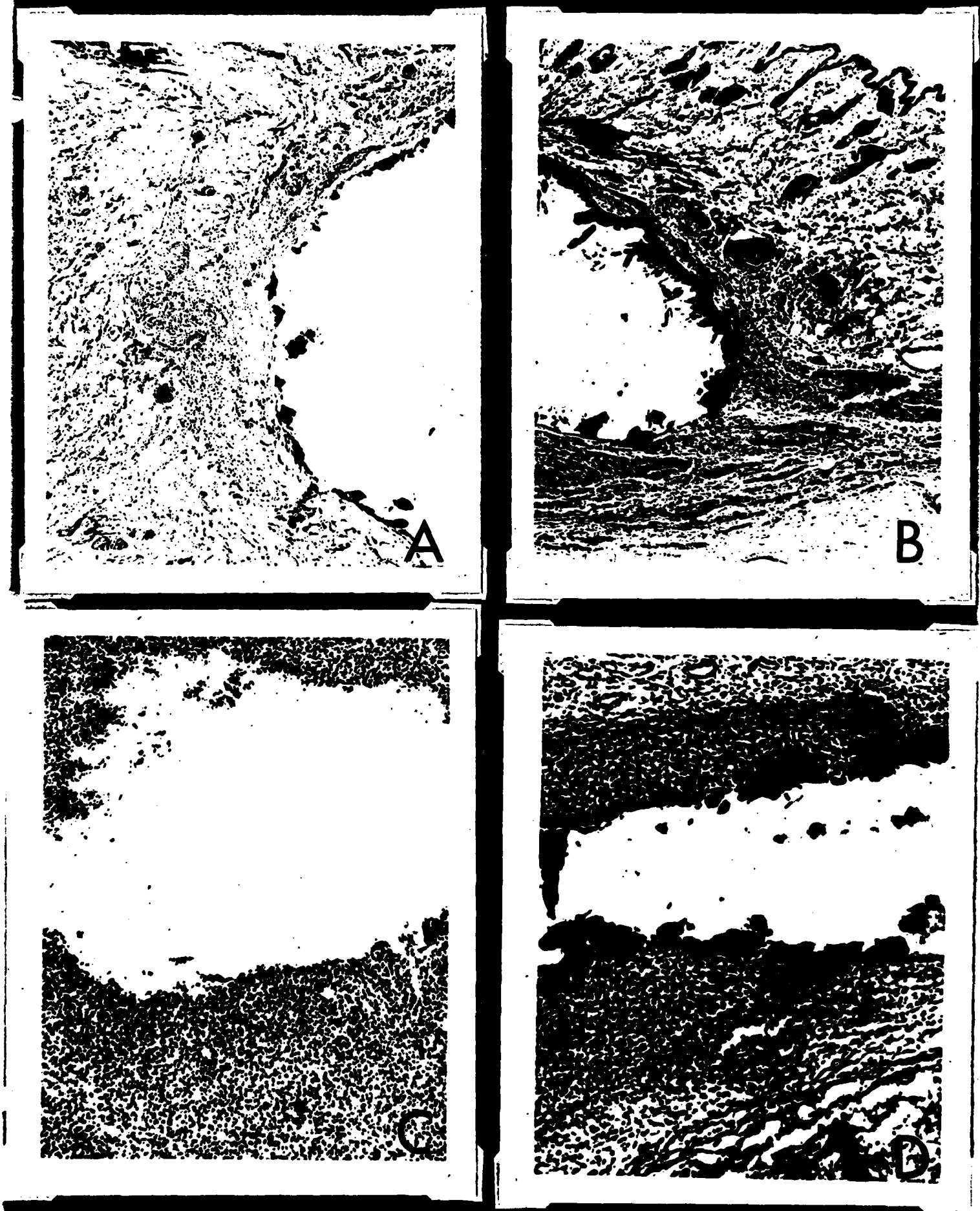

INFLAMMATION vs TIME
4
3
2
1
0
2
6
10
15
DAYS
COMMERCIAL ZOE
PURIFIED ZOE
COMMERCIAL EUGENOL
PURIFIED EUGENOL

Fig 2


A
B
C
D