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Immunological response to four pulpal medicaments

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Abstract

The purpose of this study was to test the immunological response, in an animal model, of four drugs employed in the pulpal treatment of primary teeth. The four medicaments were formalin, glutaraldehyde, sodium hypochlorite and absolute ethanol. Results indicated that both the formalin- and glutaraldehyde-treated proteins produced very mild immunological responses when an adjuvant was employed. The proteins treated with absolute ethanol, sodium hypochlorite and control saline demonstrated no such immune reactions.

Introduction

Recently, a variety of medicaments that are used in endodontic procedures have come under scrutiny because of their potential systemic effects.[1-5] Several studies have demonstrated that drugs placed in either the pulp chamber or canals do not stay confined to these areas.[1-3,6] The distribution of these drugs in laboratory animals has been shown to be widespread including the plasma, lymph nodes, skeletal muscle, heart, lungs, liver, spleen and kidneys.[1-3] It has also been shown that some of these same drugs can elicit both localized and systemic immunological responses.[4,5,7-11]

There are several possibilities that may occur when a host is exposed to an immunogenic drug and the reticuloendothelial system is stimulated. A cell-mediated response (allergic reaction) or a humoral immume response could be initiated at antigenic sites associated with the drug.[12] Alternatiavely, the drug may interact with native protein to expose new antigenic sites on the protein surface. An immune response could be generated against this newly exposed protein antigenic determinant. This response may be directed towards the newly exposed antigen or be cross-reactive with the native unaltered protein.[13,14] This latter circumstance is known as an auto-immune response. Auto-immune responses have been shown to play contributing roles in cases of rheumatoid arthritis and progressive glomerulonephritis or renal failure.[15,16,17]

Therefore, in choosing a medicament for pulpal treatment of primary teeth, consideration should be given to the following:

1. the ability to maintain the remaining pulpal tissue either inert or in a non-irritating state (usually fixation);
2. the diffusibility outside the confines of the tooth; and
3. the immunogenic potential.

It was the purpose of this study to test, quantify and compare the immunological response of four drugs employed in the pulpal treatment of primary teeth.

Methods and Materials

Animal Model

Ten female, New Zealand White rabbits[a] of approximately two kilograms each, were divided into five groups of two, and housed individually.

Antigenic Materials

Rabbit serum albumin (RSA)[b] was mixed with phosphate buffered saline pH 7.2[c] to give 2% solutions (20 mg per ml). The albumin solutions were combined with equal volumes of one of the four following endodontic medicaments plus a saline control:

1. formalin,[d] 19% in phosphate buffered saline;
2. absolute ethanol U.S.P.;[e]
3. sodium hypochlorite,[f] 2.62% in phosphate buffered saline;

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[a] Franklin Labs, Wake Forest, North Carolina.
[b] Fraction V powder, lot #4763965, Sigma Chemical Co., St. Louis, Missouri.
[c] GIBCO, Grand Island, NY.
[d] Sigma Chemical Co., St. Louis, Missouri.
[e] U.S. Industrial Chemicals Co., New York, New York.
[f] Clorox Co., Oakland, California.

4. glutaraldehyde,[g] 5% in phosphate buffered saline; and,
5. normal phosphate buffered saline.

The albumin-medicament solutions were gently mixed for five minutes at 37°C. Each was extensively dialyzed at 4°C against normal saline to remove any unbound therapeutic agent. After dialysis the solutions were adjusted to a concentration of 1% (10 mg of rabbit albumin/ml) with normal phosphate buffered saline, pH 7.2. Samples were sonicated for 15 minutes in the presence of glass beads to insure suspension of the treated rabbit serum albumins. Aliquots of the five treated rabbit albumins, which were to act as antigens, were emulsified with complete Freund adjuvant as described by Garvey, Cremer and Sussdorf.[18]

Bleeding and Injection Schedule

Each of the rabbits was bled initially for control sera on day number one. This procedure was followed by subcutaneous injections of the various potential antigens emulsified in complete Freund adjuvant. Each posterior dorsal quadrant of the back was injected subcutaneously with 7.5 mg RSA (1.5 cc emulsion) for a total of 15 mg RSA per animal.

On day 14, each of the five groups received bilateral subcutaneous booster injections of 10 mg (1.0 cc) of the appropriate antigen without the Freund adjuvant for a total of 20 mg RSA. On day 56, booster injections were given to duplicate the 14 day booster injection. At day 63, the five groups of rabbits were bled for sera for initial assay. At day 70, all rabbits were bled for final assay.

Assay for Presence of Antibodies

Ouchterlony Gel Diffusion: Ouchterlony gel diffusion was used to determine antibody titers in serum obtained from the immunized rabbits. The gel diffusion technique was completed according to the method of Garvey et al.[19] Serial dilutions of both experimental and control antisera were treated to determine antibody titers and cross reactivity between test antigens.

Passive Hemagglutination: Passive hemagglutination assays were used to determine more precisely the antibody levels in the serum obtained from the experimental animals. The procedure used was described by Garvey et al.,[20] and was modified to a micromethod. Briefly, reagents were prepared by Garvey's method and final volumes were reduced to accommodate a 96 well, round-bottom microtiter plate. The final volume was 0.2 ml.

Skin Tests: Each immunized rabbit was given four subcutaneous injections consisting of 0.5, 1.0, 1.5, and 2.0 mg of treated RSA to which it had been previously exposed. One control rabbit, which had received no treated RSA, was subcutaneously injected with 0.5 mg of each of the four treated RSA's (formaldehyde, glutaraldehyde, sodium hypochlorite and ethanol). These injections were to test for an immediate humoral response (Arthus reaction) and/or a delayed type hypersensitivity (24-72 hour) which is associated with cell-mediated immunity. The injection sites were observed at two and four hours for local swelling, erythema and punctate hemorrhages associated with an immediate humoral response. These same sites were also observed at 24, 48 and 72 hours for delayed reactions which are not only later-appearing but more indurated.[21]

[g]Lot #G6257, Sigma Chemical Co., St. Louis, Missouri.

Results

The Ouchterlony gel diffusion assay indicated that low titers of antibody were present in serum obtained from animals immunized with formaldehyde and glutaraldehyde treated RSA (Table 1). Ethanol treated, sodium hypochlorite treated, and untreated RSA did not show precipitin bands. There was no cross-reactivity between glutaraldehyde treated RSA antiserum and either formaldehyde treated RSA, or untreated RSA. The same was true for the formaldehyde treated RSA antiserum.

Serum titers obtained from passive hemagglutination assays were more quantifiable than those obtained by the gel diffusion method. However, the pattern of reactivity remained unchanged with the formaldehyde treatment demonstrating at least a twofold level of reactivity as compared to the glutaraldehyde treatment (Table 2).

Skin test results demonstrated that none of the variously treated RSA concentrations produced an

Table 1. Antibody response to treated RSA as determined by Ouchterlony gel diffusion.

Treatment	Animal	Titer[1]
Formaldehyde (19%)	A	1:2
	B	1:2
Gluteraldehyde (5)	C	1:1
	D	1:1
Ethanol (Absolute)	E	None
	F	None
Sodium Hypochlorite (2.62%)	G	None
	H	None
Control (Untreated RSA)	I	None
	J	None

[1]Highest dilution showing precipitation band.

Table 2. Antibody response to treated RSA as determined by passive hemagglutination.

Treatment Agent	Animal	Titer[1]
Formaldehyde (19%)	A	1:64
	B	1:128
Gluteraldehyde (5%)	C	1:16
	D	1:32
Ethanol (Absolute)	E	None
	F	None
Sodium Hypochlorite (2.62%)	G	None
	H	None
Control (Untreated RSA)	I	None
	J	None

[1]Highest titer showing positive agglutination.

immediate immunogenic response. The glutaraldehyde and formaldehyde treated rabbits did develop delayed mild T-cell reactions at 48 hours to their respectively treated RSA. The sodium hypochlorite, ethanol, and saline treated rabbits did not demonstrate a delayed response. In one non-immunized rabbit given all four treated albumins, no immediate or delayed responses were observed.

Discussion

In this study, the rabbit model was chosen because of its proven capability to produce high serum antibody titers to a wide range of antigens.[7,10,11] The rabbit model also afforded significant amounts of serum to be obtained while being easily maintained.

The technique of treated, purified rabbit serum albumin offered an advantage in testing for antibody titers to a single specific protein. If a combination of tissues (i.e., pulp tissues) are treated with a medicament, it is difficult to specify exactly which protein complex will demonstrate an immunogenic response since multiple combinations might be possible. In an attempt to identify and compare the immunological differences of the four pulpal medicaments, the use of the purified RSA eliminated a possible variable.

The Freund adjuvant was employed to increase the immune response of the soluble protein albumin. This increase is due primarily to a persistent slow release of antigen and the recruitment of ancillary cells to the injection sites. The ancillary cells are able to amplify the immune response. When Freund adjuvant is used with milligram quantities of antigen, as in these experiments, a specific increase in the humoral immune response is expected.[22]

The data in Tables 1 and 2 demonstrate that only the formaldehyde and glutaraldehyde treated RSA produced antibody titers. This supports the research of Horsfall,[23] Jacobs,[24] Nishidi,[8] Block,[4,5,9,25] and Thaden van Velzen[10,11] in the area of antigenic potentials of formaldehyde or glutaraldehyde containing compounds. The antibody titers obtained from the formaldehyde and glutaraldehyde treated RSA were, however, considered to be at a low level. In fact, in earlier experiments (unpublished) using the same methodology presented here, but without the Freund adjuvant, no antibody titers could be elicited from either the formaldehyde or glutaraldehyde treatments.

The results of the Ouchterlony gel diffusion assay also demonstrated that no cross reactivity existed between the antiserum to glutaraldehyde treated RSA, and either the formaldehyde treated RSA or untreated RSA. This same relationship was also found for the antiserum to formaldehyde treated RSA, and either glutaraldehyde treated RSA or untreated RSA. The possibility that the immunized animal produced significant amounts of antibody that cross-reacted with normal RSA could not be demonstrated. If the antiserum had reacted with RSA *in vivo,* it would not be possible to detect any antibody *in vitro.* Such antibody would be absorbed *in vivo* by the large quantity of RSA present in the plasma and therefore would not be detected in the *in vitro* assay system. Two findings argue against this, however:

1. The animals were healthy and showed no overt signs of auto-immune disease, such as weight loss or body wasting during or after the immunization period.
2. There was no reaction in the immediate hypersensitivity skin test (Arthus type reaction). Immediate hypersensitivity reactions require high titer antibody. Therefore, the lack of an immediate response was probably due to the low titer.

Ethanol has two possible effects on tissue protein. Absolute ethanol can fix tissue by the process of dehydration; at a lower concentration it can cause coagulation of the protein.[26,27] Either of these alterations of the RSA probably did not change the protein enough to reveal any new antigenic sites.

The sodium hypochlorite affects protein in a different manner. This reagent has the ability to denature and solubilize the protein into small molecular-weight components. If the protein was broken into amino acid residues which no longer resembled the original protein, there would be no immunologic response. Small amino acid residues are probably not immunogenic although they may still have intact antigenic sites.

Under our experimental conditions, ethanol and sodium hypochlorite treated tissues demonstrated no antigenic potential as indicated in the results. The

formaldehyde demonstrated twice the ability to produce antibody titers as glutaraldehyde, but both would still be considered to have low immune response capability at both the humoral and cell mediated levels. Since Freund adjuvant was necessary to elicit this low level immunological reaction in the animal model, it could be hypothesized that the clinical use of either formaldehyde or glutaraldehyde for pulpotomies would produce a limited, and probably insignificant, immune response. This conclusion supports the earlier work of Thaden van Velzen for glutaraldehyde and formaldehyde fixed tissues that were reimplanted.[10,11]

The remaining two considerations of diffusibility and pulp tissue maintenance have been addressed to some extent in previous literature. The latter issue (in primary teeth) has evolved into the popular use of drugs that would mummify the remaining tissue in order to render it free of clinical symptoms. The most widely advocated drug to accomplish this fixation has been formalin-containing preparations such as formocresol.[28-34] Traditionally, the formaldehyde-creosote combination has been clinically successful, but recently, formaldehyde alone has been found equally or more effective.[35,36]

The reaction of formaldehyde with protein is slow, but is optimized by a pH of 7.5 to 8.0.[37] The side group amino acids of protein react primarily with the single reaction sites of formaldehyde, thus making further enzymatic degradation difficult because of blocking of the reactive sites.[27] Similarly, intra- and inter-molecular bonds are established to stabilize the remaining protein. This entire process is in equilibrium, and therefore, the blocking of protein groups can be temporary.[35,38-40] This potential temporary fixation may be one of the explanations, along with the concentration of formaldehyde, for the various reports of either lack of fixation of portions of the pulpal tissue, or connective tissue ingrowth through the apical foramen reported in other studies.[28,41-43]

Glutaraldehyde has long been considered an excellent fixative agent for biological purposes.[44] Glutaraldehyde, like formaldehyde, predominantly fixes protein by affecting the free amino groups. However, because glutaraldehyde is bifunctional and polymeric, it has the ability to quickly and irreversibly form long molecules with effective cross linking.[38,40,45,46] The stability, therefore, of the glutaraldehyde-fixed tissue appears much greater than tissue fixed with formaldehyde. This rapid and more complete fixation, as well as stability, would be advantageous in clinical situations. Glutaraldehyde apparently causes no inflammation when used in treatment of vital and nonvital pulps of humans.[47] In fact, Hannah found that a combination of glutaraldehyde and calcium hydroxide was an excellent pulpotomy medicament when used on human permanent teeth.[48]

There has been considerable research dealing with the diffusibility of pulp medicaments from the confines of the tooth. Distribution of ^{14}C labeled formaldehyde of formocresol outside the tooth has been documented by both Myers[1,3] and Lewis.[2] Dankert has also demonstrated diffusion of formaldehyde through dentin and cementum using an antibacterial technique.[6] Glutaraldehyde did not exhibit this ability to diffuse or leach out of the tooth according to data by both Dankert[6] and 's-Gravenmade.[38] The explanation for this is not apparent but may be due to the larger bifunctional molecule of glutaraldehyde, the production of longer polymeric chains, and/or its irreversibility.[40]

Conclusion

From the results of this study the following may be elicited: 1. glutaraldehyde and formaldehyde treated RSA demonstrated a low level of antigenicity in the rabbit model; 2. formaldehyde produced a greater humoral immunological response than glutaraldehyde; 3. both formaldehyde and glutaraldehyde elicited a weak cell-mediated immune response; and 4. ethanol, sodium hypochlorite, and saline treated protein demonstrated no immunological response in the rabbit model.

This study indicates that both formaldehyde and glutaraldehyde do not stimulate a significant immune response when reacted with homologous protein. If the reaction to pulp tissue is analogous to that of homologous protein, then these agents are acceptable pulp medicaments in regard to immunogenic potentials.

These findings, plus other recent research, indicate that further investigation using glutaraldehyde as a pulp medicament is necessary. Glutaraldehyde may have the potential as an efficacious pulpotomy medicament.

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References

1. Myers, D. R., Pashley, D. H. and Whitford, G. M.: Systemic absorption of ^{14}C-Formocresol from pulpotomy sites, *J Dent Res,* 57:287, Abstract #849, January, 1978.
2. Lewis, R. D., Coffey, J., Block, R. M. and Hush, J.: Systemic distribution of C^{14} paraformaldehyde incorporated within formocresol following pulpotomy in dogs, *J Dent Res,* 57:287, Abstract #850, January, 1978.
3. Myers, D. R., Shoaf, H. K., Dirksen, T. R., Poshley, D. H., Whitford, G. M. and Reynolds, K. C.: Distribution of ^{14}C-formaldehyde after pulpotomy with formocresol, *J Am Dent Assoc,* 96: 805-813, May, 1978.
4. Block, R. M., Lewis, R. D., Sheats, J. B. and Fawley, J.: Cell-mediated immune response to dog pulpal tissue altered by cam-

phor paramonochlorophenol, *J of Pedo,* 3:303-315, Summer, 1979.
5. Block, R. M., Lewis, R. D., Sheats, J. B. and Burke, S. H.: Antibody formation to dog pulp tissue altered by N2-type paste within the root canal, *J of Endo,* 3:309-315, August, 1977.
6. Dankert, J., 's-Gravenmade, D. J. and Wemes, J. C.: Diffusion of formocresol and gluteraldehyde through dentin and cementum, *J of Endo,* 2:42-46, February 1976.
7. Okada, H., Ano, M., Yoshida, M., Munemoto, K., Nishida, O. and Yokomizo, I.: Experimental study on focal infection in rabbits by prolonged sensitization through dental pulp canals, *Arch Oral Biol,* 12:1017-1034, 1967.
8. Nishida, O., Okada, H., Kawagoe, K., Tokunaga, A., Tanchata, H., Ano, M. and Yokomizo, I.: Investigation of homologous antibodies to an extract of rabbit dental pulp, *Arch Oral Biol,* 16: 733-739, 1971.
9. Block, R. M., Lewis, R. D., Sheats, J. B. and Burke, S. H.: Antibody formation to dog pulp tissue altered by 6.5 percent paraformaldehyde via the root canal, *J of Pedo,* 3:3-15, Fall, 1977.
10. Thaden van Velzen, S. K. and Fellkamp-Vroom, T. M.: Immunologic consequences of formaldehyde fixation of autologous tissue implants, *J of Endo,* 3:179-185, May, 1977.
11. Thaden van Velzen, S. K. and van den Hooff, A.: Long-term results of the implantation of glutaraldehyde-fixed tissue, *Oral Surg,* 44:792-798, November, 1977.
12. Kimura, Y.: Focal infection and collagen disease, *Clinical Round,* 15:1879-1882, 1966.
13. Robinson, M. F., Roberts, J. L., Jones, J. V. and Lewis, E. L.: Circulatory immune complex assays in patients with lupus and membranous glomerulonephritis, *Clin Immunol and Immunopath,* 14:348-360, November, 1979.
14. Abrass, C. K., Nies, K. M., Louie, J. S., Border, W. A. and Glassock, R. J.: Correlation and predictive accuracy of circulatory immune complexes with disease activity in patients with systemic lupus erythematosus, *Arthritis and Rheumatism,* 23:273-282, March, 1980.
15. Papaioannou, C. C., Gupta, R. C., Hunder, G. G. and McDuffie, F. C.: Circulating immune complexes in giant cell arteritis and polymyalgia rheumatica, *Arthritis and Rheumatism,* 23:1021-1025, September, 1980.
16. Eagen, J. W., Roberts, J. L., Swartz, M. M. and Lewis, E. J.: The composition of pulmonary immune deposits in systemic lupus erythematosus, *Clin Immunol and Immunopath,* 12:204-219, February, 1979.
17. Sakane, T., Steinberg, A. D. and Green, D.: Studies of immune functions of patients with systemic lupus erythematosus, *Arthritis and Rheumatism,* 23:225-231, February, 1980.
18. Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.: Adjuvant-modified antigens, in *Methods in Immunology,* Third Edition, Mass: W. A. Benjamin, Inc., 1977, pp 184-185.
19. Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.: Gel Diffusion, in *Methods in Immunology,* Third Edition, Mass: W. A. Benjamin, Inc., 1977, pp 313-321.
20. Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.: Hemagglutination-passive technique with tanned erythrocytes, in *Methods in Immunology,* Third Edition, Mass: W. A. Benjamin, Inc., 1977, pp 356-360.
21. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S. and Wood, W. B.: Antibody-mediated (immediate-type) hypersensitivity, in *Immunology,* Second Edition, Maryland: Harper and Row, 1973, p 546.
22. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S. and Wood, W. B.: Cell-mediated hypersensitivity and immunity, in *Immunology,* Second Edition, Maryland: Harper and Row, 1973, pp 560-561.
23. Horsfall, F. L.: Formaldehyde and serum proteins; their immunologic characteristics, *J Immunol,* 27:553, December, 1934.
24. Jacobs, J. L. and Sommers, S. C.: Specificity of formalized proteins, *J Immunol,* 36:531, June, 1939.
25. Block, R. M., Sheats, J. B., Lewis, R. D. and Fawley, J.: Cell-mediated immune response to dog pulp tissue altered by N2 paste within the root canal, *Oral Surg,* 45:131-142, January, 1978.
26. Morton, H. E.: Alcohols, in *Disfection, Sterilization and Preservation,* ed. Block, S. S., Philadelphia: Lea and Febiger Co., 1977, pp 301-303.
27. Baker, J. A.: *Principles of Biological Microtechnique: A Study of Fixation and Dyeing,* New York: John Wiley and Sons, Inc., 1963, p 113.
28. Berger, J. E.: Pulp tissue reaction to formocresol and zinc oxide-eugenol, *J Dent Child,* 32:13-28, 1965.
29. Sweet, C. A.: Procedure for teatment of exposed and pulpless deciduous teeth, *J Am Dent Assoc,* 17:1150-1153, June, 1930.
30. McDonald, R. E. and Avery, D. R.: *Dentistry for the Child and Adolescent,* Third Edition, Missouri: C. V. Mosby Co., 1978, p 159.
31. Finn, S. B.: *Clinical Pedodontics,* Fourth Edition, Philadelphia: W. B. Saunders Co., 1973, p 211.
32. Doyle, W. A., McDonald, R. E. and Mitchell, D.: Formocresol versus calcium hydroxide in pulpotomy, *J Dent Child,* 29:87-97, 1962.
33. Massler, M. and Mansukhani, N.: Effects of formocresol on the dental pulp, *J Dent Child,* 27:277-297, Fourth Quarter, 1959.
34. Frankl, S.: Pulp therapy in pedodontics, *Oral Surg,* 34:293-309, August 1972.
35. Mejare, I. and Larsson, A.: Short-term reactions of human dental pulp to formocresol and its components — a clinical experimental study, *Scand J Dent Res,* 87:331-345, October, 1979.
36. Ranly, D. M. and Fulton, R.: Reaction of rat molar pulp tissue to formocresol, formaldehyde and cresol, *J of Endo,* 2:176-181, June, 1976.
37. French, D, and Edsall, J. T.: The reactions of formaldehyde with amino acids and proteins, in *Advances in Protein Chemistry,* eds. Anson and Edsall, Volume 2, New York: Academic Press, 1945, pp 277-235.
38. 's-Gravenmade, E. J.: Some biochemical considerations of fixation in endodontics, *J of Endo,* 1:233-237, July, 1975.
39. 's-Gravenmade, E. J. and Wemes, J. C.: Interaction of glutaraldehyde with biologic materials in endodontics, *J Dent Res,* 52: 601, Abstract #47, June, 1973.
40. Hopwood, D.: Fixatives and fixation: a review, *Histochem J,* I: 323-360, 1969.
41. Mejare, I., Hasselgren, G. and Hammarstrom, L. E.: Effect of formaldehyde-containing drugs on human dental pulp evaluated by enzyme histochemical technique, *Scand J Dent Res,* 84:29-36, 1976.
42. Beaver, H. A., Kopel, H. M. and Sabes, W. R.: The effect of zinc oxid-eugenol cement on a formocresolized pulp, *J Dent Child,* 33:381-396, 1966.
43. Emmerson, C. C., Miyamoto, O., Sweet, C. A. and Bhatic, H. D.: Pulpal changes following formocresol applications on rat molars and human primary teeth, *J S Cal Dent Assn,* 27:309-323, 1959.
44. Sabatine, D. S., Bensch, K. and Barrnett, R. J.: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixative, *J Cell Biol,* 17:19-58, April, 1963.
45. Richards, F. M. and Knowles, J. R.: Glutaraldehyde as a protein cross-linking reagent, *J Mol Biol,* 37:231-233, 1968.
46. Habeeb, A. F. S. A. and Heramoto, R.: Reaction of proteins with glutaraldehyde, *Arch Biochem Biophys,* 126:16-26, 1968.
47. Wemes, J. C. and 's-Gravenmade, E. J.: Glutaraldehyde: a new fixative in endodontics, *J Dent Res,* 52:601, Abstract #48, June, 1973.
48. Hannah, D. R.: Glutaraldehyde and calcium hydroxide: a pulp dressing materials, *Brit Dent J,* 132:227-231, March, 1972.