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FIBROSING PROPERTIES, IN THE RAT, OF COMPOUNDS RELATED TO DICETYL (DI-*n*-HEXADECYL) PHOSPHATE—I*

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Abstract—In this preliminary survey, twenty-six compounds, related in one way or another to dicetyl(di-*n*-hexadecyl)phosphate, were compared with this substance as to tissue fibrosing activity by subcutaneous injections in the rat. Only dialkylphosphates (and the single alkylborate used) with a chain length of sixteen or eighteen carbon atoms were active. The only substance with unsaturated alkyl chains, dioleyl(C_{18})phosphate, was inactive. Dicholesteryl-phosphate and phosphite were not active. None of the phosphites (di- or tri-) was active. Dicetylborate produced less fibrosis than the phosphate and this was accompanied by marked eosinophilia and subsequent encapsulated necrosis. Dioctadecyl phosphate was indistinguishable in these experiments from dicetyl phosphate.

USE of sclerosing compounds has long been an accepted method of producing fibroblastic activity in both experimental and clinical medicine. This has been extended from early attempts merely to obliterate varicose veins to a much more diversified use today.

Besides their injection into varicose veins,[1–6] and most certainly less well known, has been the use of materials which initiate fibroblastic activity, in order to obliterate ganglia of the wrists,[7] angiomata,[8] fistulae,[9] hemorrhoids,[10] umbilical herniae,[11] inguinal herniae,[12] idiopathic hydrocele,[13] verrucae plantaris,[14] blood vessels and aneurysms.[15–20] These substances have also been used in situations in which physiology or anatomy is intentionally altered. Among these are the treatment of urinary incontinence,[21] experimental nerve sclerosing,[22] obliteration of the adrenal medulla,[23] and the strengthening or replacement of ligaments and tendons.[24, 25]

Compounds or materials used in these procedures have been as widely diversified as the procedures themselves and have included cellophane or polyethylene,[15–17, 25–31] sodium morrhuate,[20] aspirin,[23] sodium tetradecyl sulfate,[1, 14, 22] and sodium dicetyl (di-*n*-hexadecyl) phosphate.[18, 19, 21, 22]

Interest was stimulated in pure compounds as sclerosing agents when Page[26] produced hypertension in dogs by wrapping their kidneys with cellophane. Poppe

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and De Oliveira[17] then studied various types of cellophane, some producing marked fibroblastic activity and some being relatively inert. Several reports followed, giving uses of cellophane or polyethylene in an inert capacity,[25, 32–34] while others appeared describing the fibroblastic activity of these polymers.[16, 27] In an excellent historical review Yeager and Cowley[35] showed that while polyethylene itself was inert, dicetyl phosphate, sometimes used in the processing of these plastic sheets, was a potent fibroblastic stimulant. Cooper *et al.*[28] also arrived at the same conclusion. Since then some attention has been directed toward dicetyl phosphate itself as a fibrosing agent, especially for the treatment of aneurysm.[19a, b]

The present study was initiated in order to examine the fibrosing properties of substances related structurally, in one way or another, to dicetyl phosphate. Other than Reiner's observations[20] regarding some soaps, synthetic detergents, sodium ricinoleate and sodium morrhuate, there seems to be no systematic examination in the literature for structure–activity relationships of any series of fibrosing compounds. We felt it, therefore, of intrinsic interest to find the structural limitations for fibrosing activity in this series. We hoped, also, to gain increased knowledge of the mechanism by which these substances promote formation of fibrous tissue. In addition, there was the possibility of finding a substance more readily applicable and more rapid in its action than dicetyl phosphate.

METHODS

In this initial survey twenty-six compounds (Table 1) related to dicetyl phosphate were tested in comparison with the latter substance. A number of these were synthesized in this laboratory and some are new to the literature. The preparation and chemical properties of the latter will be described in a paper now in preparation in which we will further extend data on the structural requirements for fibrosing activity in this series.

All compounds were given to Wistar rats by subcutaneous injection, using groups of ten animals (male and female) for each substance. The compounds were either dissolved or suspended (5 per cent) in isotonic saline or (10 per cent) in corn oil.*

TABLE 1. SUBSTANCES INVESTIGATED FOR POSSIBLE TISSUE-FIBROSING ACTION, AS COMPARED WITH DICETYL PHOSPHATE

Compound	Formula	Footnote
(1) Didecylphosphate	$(C_{10}H_{21}O)_2\,P(=O)OH$	*
(2) Diundecylphosphate	$(C_{11}H_{23}O)_2\,P(=O)OH$	*

* Suspensions of the long-chain phosphates were made by heating them in corn oil on the water bath, at not over 80 °C, for a short time until they were in solution. They were then allowed to cool and injected at not over 40° as a gel-like mixture.

TABLE 1—*continued.*

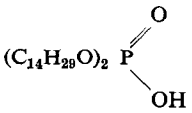
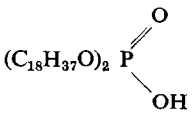
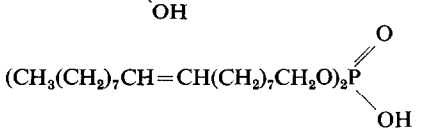
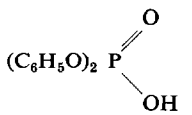
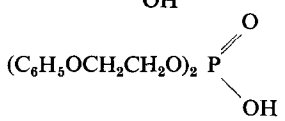
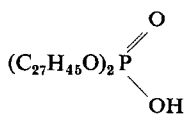
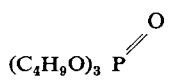
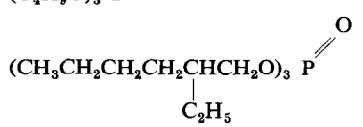
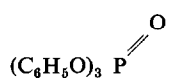
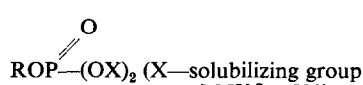
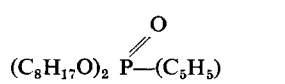
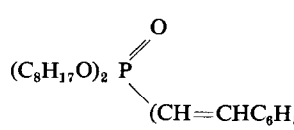
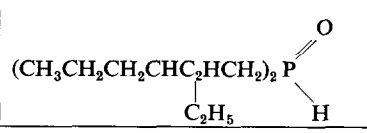
Components	Formula	Footnote
(3) Dimyristyl phosphate		†
(4) Dioctadecyl phosphate		†
(5) Dioleyl phosphate (crude)		*, †, (a)
(6) Diphenyl phosphate		
(7) Diphenoxyethyl phosphate		*
(8) Di (or pyro-) cholesteryl phosphate		§
(9) Tributyl phosphate		‡, (b)
(10) Tri-2-ethylhexyl phosphate		‡, (c)
(11) Triphenyl phosphate		
(12) "Victawcet-14"		‡, (d)
(13) Di*iso*octylphenyl phosphonate		‡, (d)
(14) Di*iso*octylstyryl phosphonate		‡, (d)
(15) Di-2-ethylhexyl phosphite		†, (e)

TABLE 1—*continued.*

Compound	Formula	Footnote
(16) Dicetyl phosphite	$(C_{16}H_{33}O)_2\,P(=O)H$	*
(17) Dicholesteryl phosphite	$(C_{27}H_{45}O)_2\,P(=O)H$	**
(18) Tri (? with di) decyl phosphite	$(C_{10}H_{21}O)_2\,P(=O)H$	*
(19) Tri (? with di) lauryl phosphite	$(C_{12}H_{25}O)_2\,P(=O)H$	*
(20) Tri (? with di) myristyl phosphite	$(C_{14}H_{29}O)_2\,P(=O)H$	*
(21) Tri (? with di) octadecyl phosphite	$(C_{18}H_{37}O)_2\,P(=O)H$	*
(22) Tri-2-ethylhexylphosphite	$(CH_3CH_2CH_2CH_2CH(C_2H_5)CH_2O)_3P$	‡, (e)
(23) Triisooctylphosphite	$(C_8H_{17}O)_3P$	‡, (e), ††
(24) Triphenylphosphite	$(C_6H_5O)_3P$	
(25) Tri (? with di) cetylborate (unstable)	$(C_{16}H_{33}O)_2BOH$	*
(26) Dicetylether	$(C_{16}H_{33})_2O$	‡ (f)

* New compound.
† Made in this laboratory.
‡ These compounds were obtained through the generosity of:
(a) The oleyl alcohol was donated by M. Michel & Co., Inc., N.Y., N.Y.
(b) Commercial Solvents Corporation, N.Y., N.Y.
(c) Carbide and Carbon Chemical Corporation, N.Y., N.Y.
(d) Victor Chemical Works, Chicago 6, Ill.
(e) Virginia-Carolina Chemicals Corporation, Richmond, Va.
(f) Humphrey-Wilkinson, Inc., North Haven, Conn.
§ Awaits characterization: from the reaction of cholesterol and PCl_3 with pyridine in benzene.
** Awaits characterization: from the reaction of cholesterol and PCl_3 with pyridine in benzene.
†† Mixture of octyl isomers.

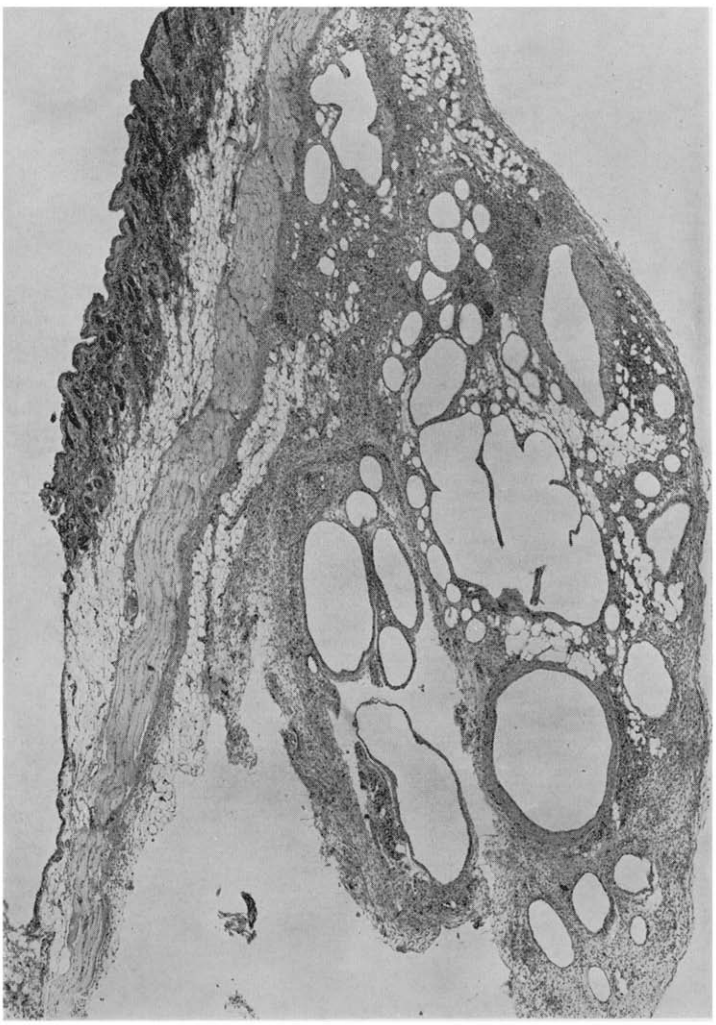


FIG. 1 (a). Control: corn oil injection site at 6 weeks showing persisting sites of corn oil vesicles. This grossly gives the appearance of small vesicular areas bounded by thin avascular walls. × 24.

facing page 234

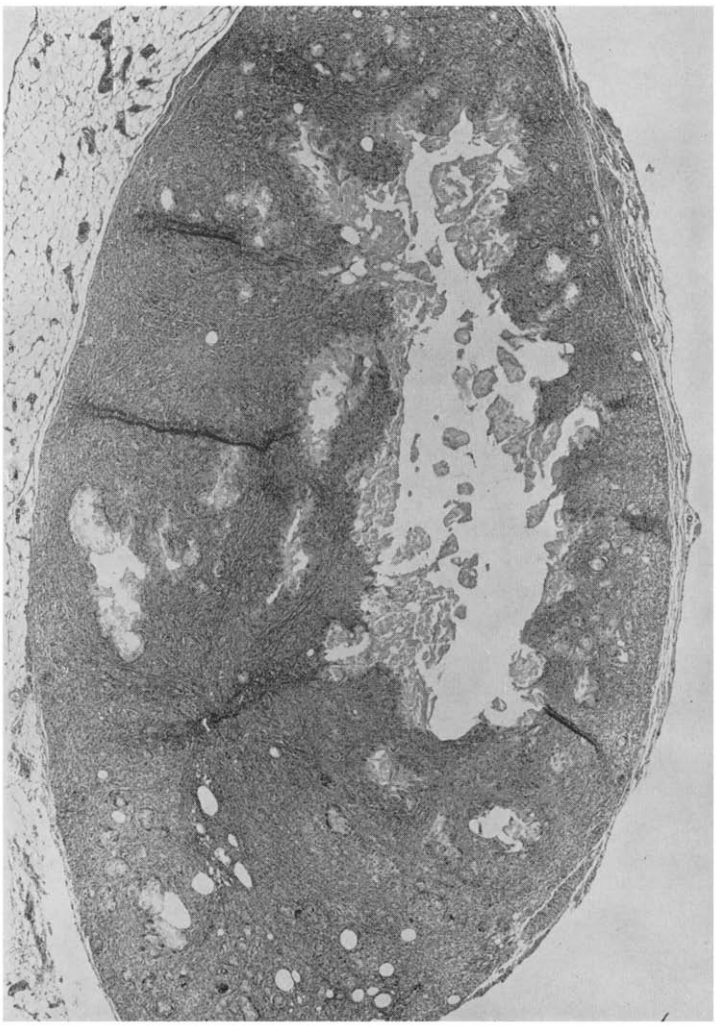


FIG. 1 (b). Dicetylphosphate in corn oil: injection site at 6 weeks, showing extensive fibrosis about small central areas of necrosis. Giant cells are appearing in great numbers throughout the densely fibrotic areas. 24.

Each compound was injected (0·1 ml) subcutaneously only once in two places (saline and corn oil) on the right abdomen.* Dicetyl phosphate was similarly injected (left abdomen) in all animals for comparison.

Following this, animals were weighed weekly for six weeks to determine toxicity. Microscopic examination was made† of all fibroblastic nodules for each active substance. One-half the surviving animals were sacrificed at 6–8 weeks and the remainder at 6 months following injection.

RESULTS AND DISCUSSION

Compounds showing no fibrosing activity in 6–8 weeks showed no change at 6 months. Active compounds had stimulated the maximum response in 6–8 weeks (or less) and there appeared to be no change at 6 months. We cannot say on the basis of present results that either corn oil or saline injections resulted in greater response to active compounds. The corn oil nodules in general appeared larger; the center of these was usually hollow and contained what seemed to be some of the original corn oil.

Other than dicetylphosphate, only two compounds, dioctadecylphosphate and di (tri?) cetylborate showed appreciable fibrosing activity. The former was approximately equal in activity to dicetylphosphate: the latter was different qualitatively, being accompanied by marked eosinophilia during the first week (see Table 2) and the presence of numerous mast cells.† Central necrosis occurred during the second week and became marked, but was contained by the fibrous capsule. None of the nodules at the sites of dicetyl or dioctadecyl phosphate injection showed appreciable necrosis. See Fig. 1 for representative illustrations.

Compound nos. 11 and 22 (Table 1) seemed to have marked toxicity. All the animals showed signs of debility a day or so after injection and died in a few weeks.

The control groups injected with saline alone had no fibrous nodules. Sites at which corn oil alone was injected developed small encapsulated vesicles with thin avascular walls. None of the latter was thickened or fibrotic.

Table 2 shows the results of an experiment comparing the three active compounds and giving a more detailed picture of the rate of fibrosis. In this experiment three groups of thirty rats each were injected as described above and five of each group were sacrificed at the end of each week.

Before attempting any serious explanation of these findings we are extending our study to include the following compounds: monoalkylphosphates, dialkylphosphates with chain length of more than eighteen carbons, tricetylphosphate, dialkylphosphates with an odd number of carbon atoms (especially diheptadecylphosphate and dipentadecylphosphate) and with branching‡ on the chain, and monocetylphosphite.

Preliminary findings in this projected work, together with the foregoing, indicate that for activity with this type of substance there must be at least one free —OH group, suggesting that enzymes which split esters of phosphoric acid may be involved. The chain must be more than fourteen carbons in length.

* The area to be injected was sponged with alcohol and sterile needles and syringes were used, otherwise no special aseptic precautions were taken and no deaths or recognizable differences were apparent which could be attributed to this aspect of the technique.

† We are indebted to Dr. R. Reiff, formerly of the Department of Pathology, for examining the specimens and slides.

‡ An example of the latter, isolated from wool wax (Australian sheep) is now in our hands.

The only unsaturated alcohol represented thus far (oleyl, C_{18}) may react in a different manner because of the double bond. The fact that the latter was a crude sample (see Table 1) with at least one other component (mono-oleylphosphate) makes it advisable that these results be confirmed before any conclusions are reached about this type.

The apparent abrupt cut-off (C_{16}, active; C_{14}, inactive; C_{15}*, ?) is not understood. If gradual diminution in activity with shortening chain length were observed, we

TABLE 2. COMPOUNDS SHOWING TISSUE-FIBROSING ACTIVITY COMPARABLE TO THAT OF DICETYLPHOSPHATE (6-WEEK PERIOD)

Week	Dicetylphosphate	Dioctadecylphosphate	Tri (?di) cetylborate
1	Marked fibrosis; little to no eosinophilia	Marked fibrosis; little to no eosinophilia	Moderate fibrosis. Extreme eosinophilia numerous tissue mast cells
2	Fibrosis increasing and penetrating to center of nodule	Fibrosis increasing and penetrating to center of nodule	Central necrosis, with little fibrosis; eosinophilia decreasing
3	Thickly fibrosed, with giant cells appearing in greater numbers	Quite comparable to reaction with dicetyl phosphate	Little fibrosis; necrosis, still some eosinophils but appearing to be a chronic inflammatory response
4	Extensive fibrosis, even into center of large nodules; slight central necrosis	Extensive fibrosis, but perhaps a little less centrally than with dicetyl phosphate	Thinly encapsulated, necrotic mass; eosinophilic response disappearing
5	Extensive fibrosis continuing, although near a maximum	Extensive fibrosis (as with dicetyl phosphate)	Thinly encapsulated necrotic mass; detritus in center of nodules
6	Extensive fibrosis throughout nodules	Extensive fibrosis throughout nodules	No additional fibrosis; necrosis continuing, with thin capsule of inflammatory reaction surrounding central detritus

could perhaps assume that greater solubility in the body fluids leads to more rapid assimilation of the smaller molecules, thus removing them from the injection site before action leading to fibrosis takes place. The interesting observation of Theorell, recently discussed,[36] regarding a similar sharp break at C_{15} (fatty acid) in the formation of ternary complexes in the liver–ADH system is provocative, although any relation between these phenomena is not apparent.

* We have recently obtained some *n*-pentadecanol through the kindness of the Cancer Chemotherapy Service Center of the U.S. Public Health Service.

REFERENCES

1. E. J. ORBACH, *J. Amer. Med. Ass.* **166**, 1964 (1958).
2. E. J. ORBACH and A. K. PETRETTI, *Angiology* **1**, 237 (1950).
3. E. J. ORBACH, *Angiology* **1**, 302 (1950).
4. W. M. COOPER, *Surg. Gynec. Obstet.* **83**, 647 (1946).

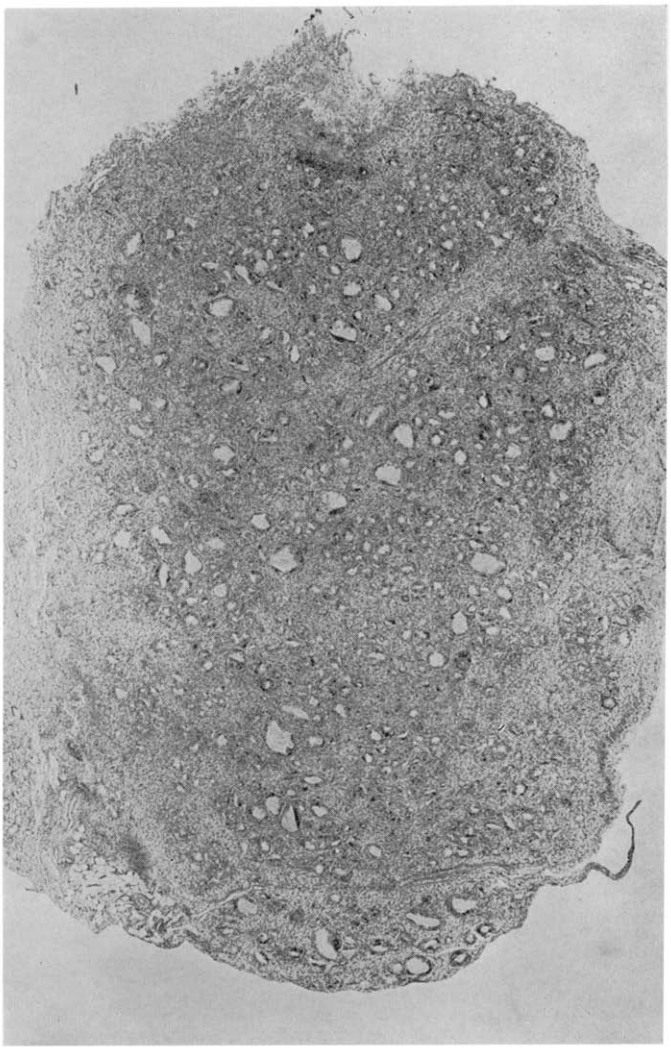


FIG. 1 (c). Dioctadecyl phosphate in normal saline: injection site at 6 weeks, showing extensive fibrosis throughout nodule with only a few limited necrotic areas. Some giant cells are present. × 24.

facing page 236

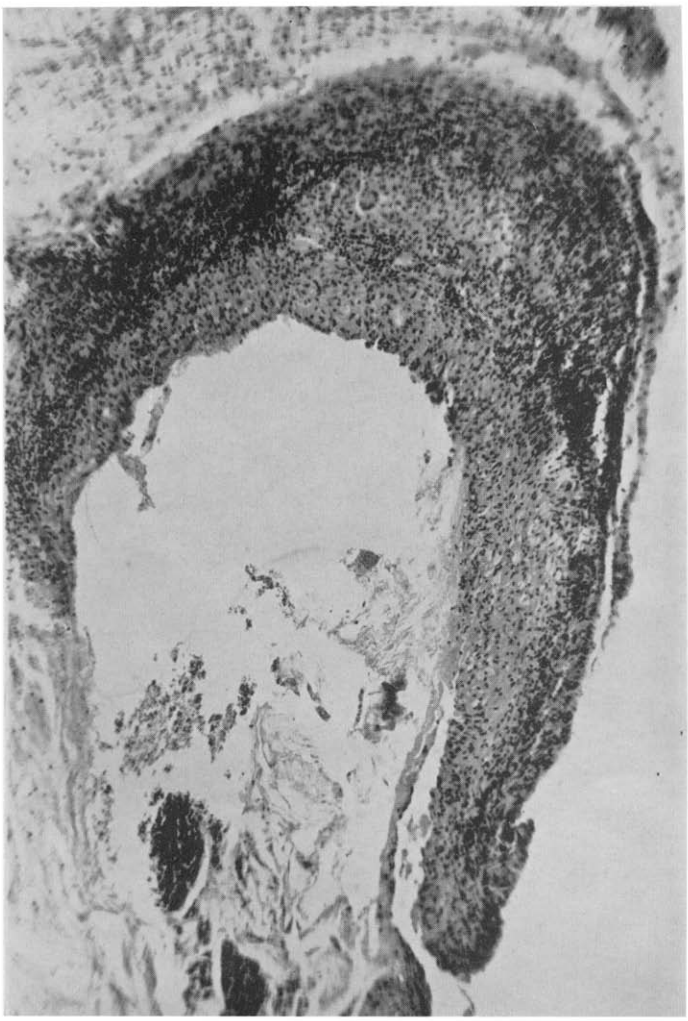


FIG. 1 (d). Dicetyl borate in normal saline: injection site at 6 weeks, showing small outer margin of fibrosis with a thin wall of inflammatory reaction about central detritus. Many eosinophils and mast cells present. × 88.

5. J. A. DINGWALL, D. T. W. LIM and J. A. LYON, *Surgery* **23**, 599 (1948).
6. H. O. MCPHEETERS and J. K. ANDERSON, *Injection Treatment of Varicose Veins and Hemorrhoids*. F. A. Davis, Philadelphia (1946).
7. V. SALEMI and C. SEBASTIANI, *Gior. Med. Mil.* **106**, 716 (1956).
8. J. R. LEWIS, JR., *Plastic Reconstr. Surg.* **19**, 201 (1957).
9. J. ERBSLÖH, *Z. Urol.* **52**, 40 (1959).
10. G. L. WRIGHT, *Rocky Mtn. Med. J.* **56**, 69 (1959).
11. L. MANNESFELDT and W. TISCHER, *Kinderärztl. Praxis* **27**, 136 (1959).
12. F. H. BAEHR, *J. Internat. Coll. Surgeons* **29**, Pt. 1 (1958).
13. J. NAYMAN, *S. Afr. Med. J.* **33**, 409 (1959).
14. L. L. CRAMER, *J. Amer. Podiat. Ass.* **48**, 526 (1958).
15. H. E. PEARSE, *Ann. Surg.* **112**, 923 (1940).
16. P. W. HARRISON and J. CHANDRY, Ann. Surg. **118**, 478 (1943).
17. J. K. POPPE and R. DE OLIVEIRA, *J. Thoracic Surg.* **15**, 186 (1946).
18. J. K. BERMAN, F. J. BOYD, JR. and W. K. SAINT, *J. Indiana Med. Ass.* **42**, 889 (1949).
19. (a) J. K. BERMAN and J. E. HULL, *Surg. Gynec. Obstet.* **94**, 543 (1952).
19. (b) R. K. ZECH *et al.*, *Angiology* **5**, 439 (1954).
20. L. REINER, *Proc. Soc. Exp. Biol., N.Y.* **62**, 49 (1946).
21. H. KLOSTERHALFEN, *Z. Urol.* **51**, 412 (1958).
22. A. J. KOSLIN, *Oral Surg.* **11**, 1404 (1958).
23. I. J. GREKOVA, *Vestnik. Khir.* **81**, 114 (1958).
24. G. J. BRONSTON, *J. Amer. Podiat. Ass.* **48**, 511 (1958).
25. T. WHEELDON, *J. Bone & Joint Surg.* **21**, 393 (1939).
26. I. H. PAGE, *J. Amer. Med. Ass.* **113**, 2046 (1939).
27. F. R. HARPER and M. E. ROBINSON, *Amer. J. Surg.* **64**, 294 (1944).
28. F. W. COOPER, R. L. ROBERTSON, P. C. SHEA, JR. and E. W. DENNIS, *Surgery* **25**, 184 (1949).
29. O. A. ABBOTT, *J. Thoracic Surg.* **18**, 435 (1949).
30. I. C. MIDDLEMAN and N. W. DREY, *Surgery* **29**, 890 (1951).
31. J. K. POPPE, *Diseases of the Chest* **15**, 726 (1949).
32. F. D. INGRAHAM, E. ALEXANDER, JR. and D. D. MATSON, *J. Amer. Med. Ass.* **135**, 82 (1947).
33. D. DONATI, *Bull. sc. med., Bologna* **109**, 425 (1937).
34. D. C. MCKEEVER, *J. Bone & Joint Surg.* **25**, 576 (1943).
35. G. H. YEAGER and R. A. COWLEY, *Ann. Surg.* **128**, 509 (1948).
36. CIBA FOUNDATION, *Tenth Anniversary Symposium; Significant Trends in Medical Research* pp. 37–39. Little, Brown, Boston (1959).