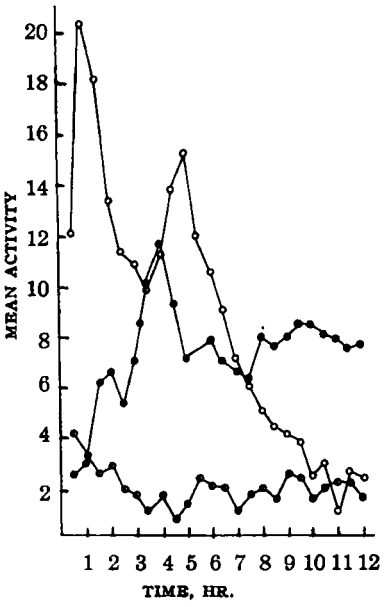

Fig. 3.—*In vivo* comparison of tableted coated and uncoated granules. Key: ● coated, O uncoated, ◐ control.

thickness, which occurs in pan coating. This was shown by the *in vivo* procedure.

An added advantage to this approach to obtaining sustained release is the use of a single coating composition which is applied in one concentration to an entire batch of granules. Therefore the entire coating process is accomplished in one easy operation without additional coatings and mixing of the coated granules.

SUMMARY

d-Amphetamine sulfate granules were successfully coated with a plasticized film of the *n* butyl half ester of PVM/MA 119 using each of three dusting powders: talc, magnesium stearate, and precipitated calcium carbonate. *In vitro* release rates were determined for each coating and the granules coated with the aid of precipitated calcium carbonate produced the most satisfactory results. *In vitro* tests indicated that the coated granules could be recovered intact from compressed tablets.

In vivo release rates were determined using the Williamson activity cage to measure spontaneous motor activity. The results obtained showed that the activity of the rats receiving the coated granules was 4.5 hours longer than those receiving uncoated granules.

In vivo analysis of tablets prepared by compressing the coated granules in a polyethylene glycol 6000 matrix, showed a continued increase in motor activity after 12 hours, while the tablets containing uncoated granules showed no increase after 7.5 hours.

REFERENCES

(1) Lazarus, J., and Cooper, J., *J. Pharm. Pharmacol.*, 11, 257(1959).

(2) Stempel, E., J. Am. Pharm. Assoc., Pract. Pharm. Ed., 20, 393(1959).

(3) Nalin, E., Yoss, E. J., Sacks, M., Sacks, I., and Smedresman, M. (to Nysco Labs), U. S. pat. 2,963,402 (Dec. 6, 1960).

(4) Wagner, J. G. (to Upjohn Co.), U. S. pat. 2,897,121 (July 28, 1959).

(5) Higuchi, T., and Kuramoto, R., This Journal, 43, 393, 399(1954).

(6) Royal, J., *Drug Std.*, 26, 41(1958).

(7) Winer, J. B., "Statistical Principles in Experimental Design," McGraw-Hill Publishing Co., New York, N. Y., 1961, p. 82.

Gas Chromatography of Some Antihistamines

By ALEXANDER MACDONALD, JR., and RONALD T. PFLAUM

The gas chromatographic behavior of 16 antihistamines was investigated using packed 0.010-in. diameter capillary and 0.065-in. open tubular columns. All packed columns were 6 ft. long, with a low phase to support ratio and were operated at 175°. The liquid phases used were Carbowax 20M, SE-30, XF-1150, and PDEAS. Of the seven solid supports evaluated for use in low loaded columns, only glass beads, Gas Chrom-P, and Chromosorb W-HMDS proved to be satisfactory. The most successful column for the separation of these antihistamines was a 6-ft.–0.08% PDEAS on 120/170 glass-bead column operated at a temperature of 175°. This column provided good separation and symmetrical peaks. Instrument limitations prevented any valid evaluation of the 0.010-in. capillary column and of the 0.065-in. open tubular column for antihistamine separation.

Gas chromatography has proved to be a valuable tool for the separation and identification of many types of compounds. In pharmaceutical analysis, barbiturates (1–4), sympathomimetic amines (5–7), and tranquilizers (8, 9) have been successfully separated by gas chromatography. Toxicological screening of alkaloids and the above classes using gas chromatographic separation on a single column has been reported by Parker (10). Gas chromatographic data on 14 antihistamines using a 5-ft. × 1/8-in. diameter–2% Carbowax 20M on 10% KOH

Received August 20, 1963, from the Department of Chemistry, State University of Iowa, Iowa City.
Accepted for publication November 21, 1963.

TABLE I.—RETENTION TIMES OF ANTIHISTAMINES ON FOUR PHASES

Compd.[d]	SE-30,[a] Min.	PDEAS,[a] Min.	XF-1150,[a] Min.	Carbowax 20M,[a] Min.
Antazoline (Ciba Pharmaceutical Co.)	...[b]	65.8	...[b]	...[b]
Carbinoxamine (McNeil Laboratories)	4.3	8.5	10.6	10.6
Chlorcyclizine (Burroughs Wellcome)	6.2	12.9	14.9	12.0
Chlorothen (Lederle Laboratories)	5.3	10.5	13.5	10.6
Chlorpheniramine (Schering Research)	3.0	5.2	6.6	6.0
Cyclizine (Burroughs Wellcome)	3.2	4.9	6.2	4.8
Diphenhydramine (Parke, Davis & Co.)	1.7	2.5	3.9	2.9
Doxylamine (Wm. S. Merrell Co.)	2.3	3.9	4.2	4.5
Meclizine (Chas. Pfizer & Co.)	N.R.[c]	N.R.[c]	N.R.[c]	N.R.[c]
Methapyrilene (Irwin Neisler & Co.)	2.8	5.8	7.7	5.5
Pheniramine (Dorsey Laboratories)	1.4	2.1	2.8	2.4
Promethazine (Wyeth Laboratories)	7.2	21.7	27.9	17.1
Pyrilamine (Dorsey Laboratories)	7.6	20.3	23.6	20.2
Thenyldiamine (Sterling Winthrop Research Institute)	2.6	6.9	9.7	6.1
Thonzylamine (Warner-Lambert Research Institute)	6.2	18.0	20.6	17.2
Tripelennamine (Ciba Pharmaceutical Co.)	2.7	5.0	6.5	5.0
Support	Glass beads	Glass beads	Chromosorb W-HMDS	Gas Chrom P
Mesh	120/170	120/170	100/120	100/120
Liquid phase, w/w%	0.07	0.08	1.07	1.08

[a] Column length, 6 ft.; column temperature, 175° C.; detector temperature, 230° C.; injection point temperature, 260° C.; argon flow rate, 60 ml./minute. [b] Slight response due to decomposition product observed. [c] No response. [d] Supplier of compound.

coated 60/80 Chromosorb W has been reported by Fontan (11).

Present interest was concerned with the gas chromatographic behavior of a series of 16 antihistamines. Optimum conditions for packed column separation of these antihistamines on four different phases were established after evaluating the effect of phase loading, solid support, and column operating conditions. These groups of antihistamines were also investigated using a 0.010-in. diameter capillary column and a 0.065-in. diameter open tubular coated column.

EXPERIMENTAL

Apparatus and Reagents.—A Barber-Colman model 10 dual column gas chromatograph equipped with an argon ionization detector and a hydrogen flame ionization detector was used in this work. All packed columns were prepared from 6-ft. glass U-tubes having an inside diameter of 3 mm. A 100-ft. stainless steel capillary column of 0.010 in. inside diameter and a 100-ft. copper open tubular column of 0.065 in. inside diameter were also used. Each of these columns was wound on a 5-ft. section of $^1/_4 \times 1^1/_2$-in. steel bar designed to fit conveniently into the column oven of the chromatograph.

Seven solid supports were investigated for use in column packings. Gas Chrom-P was obtained from Applied Science Laboratories, State College, Pa. Chromosorb W-HMDS, Fluoropak-80, and glass beads were obtained from Wilkins Instrument and Research, Inc., Walnut Creek, Calif. Gas Pack-F was obtained from Chemical Research Services, Inc., Addison, Ill. Crushed firebrick and acid washed crushed firebrick were prepared in this laboratory from G-30 insulating firebrick obtained from A. P. Green Firebrick Co., Mexico, Mo.

The following four liquid phases were used in the various columns: polyethylene glycol (Carbowax 20M, Union Carbide Chemicals Co., New York, N. Y.), methyl silicone rubber gum (SE-30, Applied Science Laboratories), nitrile silicone polymer liquid (XF-1150, Wilkins Instrument and Research), and phenyldiethanolamine succinate polymer (PDEAS, Wilkins Instrument and Research).

The 16 antihistamines were generously furnished by the pharmaceutical companies listed in Table I. All were used as received without further purification.

PROCEDURES

The column packings were prepared by adding a weighed amount of the liquid phase in a suitable solvent to a weighed amount of the solid in a 1-L. creased round bottom flask (12). The solvent was removed using a rotary evaporator and the phase coated support dried at 110° for 4 hours. The glass columns were filled by the slow addition of packing to the tube which was settled by vibration (vibrating spatula) and tapping (rubber mallet). The prepared columns were equilibrated for 12 hours at a temperature of 175° and a flow rate of 20 ml./minute.

The procedure outlined by Averill was followed in coating the capillary and open tubular column with a liquid phase (13).

Two procedures were used for the preparation of chromatographic samples. In the first, a stock solution of 0.5 Gm. of the antihistamine salt in 50 ml. of distilled water was prepared. A 5-ml. aliquot of this solution was placed in a 30-ml. separator, 1 ml. of 6 *N* NH_4OH added, and the free amine extracted with 0.5 ml. reagent grade chloroform.

In the second procedure, a weighed sample of 0.01 Gm. of the salt was dissolved in 5.0 ml. of distilled water. One milliliter of 6 *N* NH_4OH was added to the 5-ml. solution in the separator and the free amine extracted with 0.5 ml. of reagent grade chloroform.

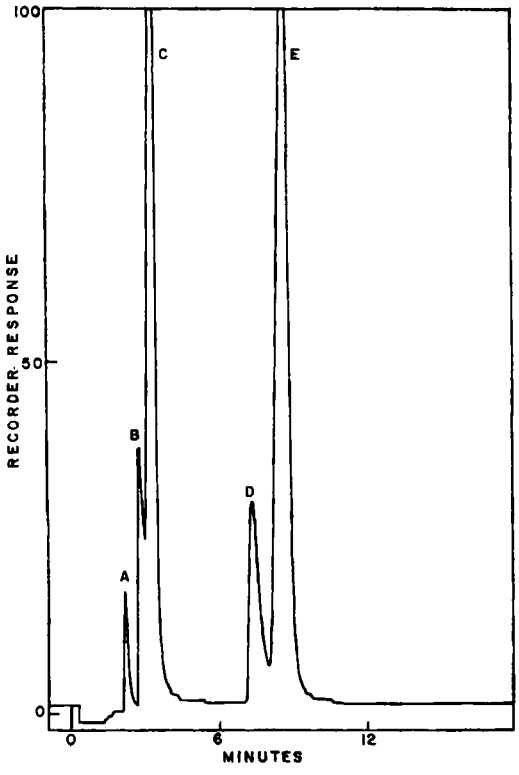

Fig. 1.—Separation using a 6-ft.—0.07% SE-30 on 120/170 glass bead column at 175° C. Key: *A*, diphenhydramine; *B*, doxylamine; *C*, methapyrilene, thenyldiamine; *D*, chlorcyclizine, thonzylamine; and *E*, promethazine, pyrilamine.

Synthetic mixtures of the antihistamines were prepared either by combination of the individual extracts or by the treatment and extraction of a mixture of the antihistamine salts.

A Hamilton 10-μl. syringe was used to inject the chloroform extract into the chromatographic column.

The behavior of several antihistamines was used to evaluate the following column parameters over the indicated ranges: liquid phase per cent (1–5%), solid supports (seven listed), column length (6 to 8 ft.), column temperature (155 to 189°), injection port temperature (185 to 260°), and column flow rate (10 to 85 ml./minute). The maximum number of theoretical plates calculated was used as the criteria to determine the optimum value for each of the above variables.

RESULTS AND DISCUSSION

Separation of the antihistamines with minimal tailing in a short analysis time was desired for each of the four liquid phases. The optimum operating conditions determined for each phase and the antihistamine retention times on them are listed in Table I. The separation obtainable with each of these columns is shown in Figs. 1–4. Figures 1–4 indicate that Carbowax 20M, PDEAS, and XF-1150 are much better phases for the separation of these compounds than SE-30. The PDEAS and XF-1150 columns offer comparable separation to the Carbowax 20M column but with a decrease in tailing. The 0.8% PDEAS on 120/170 glass-bead column was the most efficient column used because it provides adequate separation and symmetrical peak shapes. The response of the argon ionization detector, using the PDEAS on glass-bead column, was linear over the range of 1–20 mcg. of antihistamine. The minimum identifiable amount was arbitrarily chosen as the amount which would give a 10% scale unattenuated response using the argon ionization detector. The minimum amount determined for each antihistamine is shown in Table II.

Samples of antazoline yield very small peaks with short retention times on all columns, which

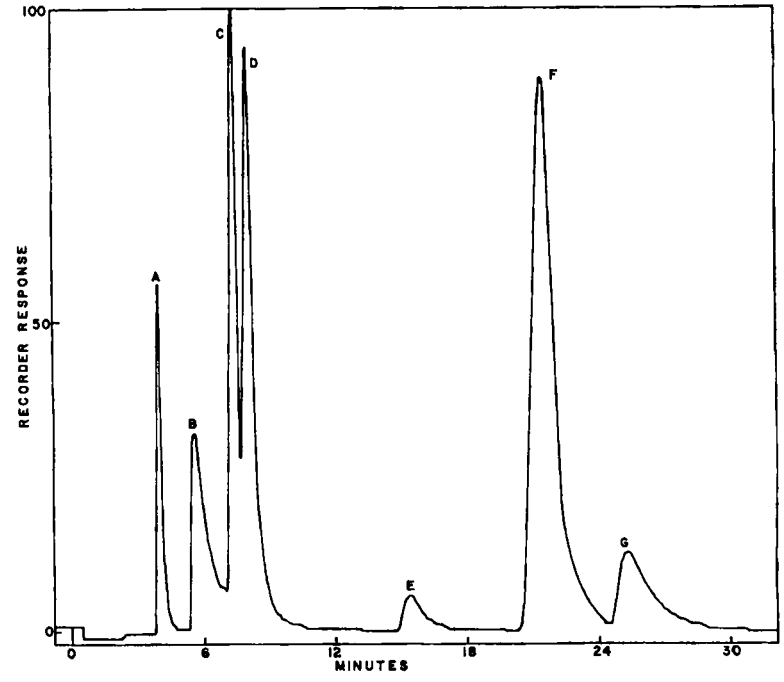

Fig. 2.—Separation using a 6-ft.—1.08% Carbowax 20M on 100/120 Gas Chrom-P column 170° C. Key: *A*, diphenhydramine; *B*, doxylamine; *C*, methapyrilene; *D*, thenyldiamine; *E*, chlorcyclizine; *F*, promethazine, thonzylamine; and *G*, pyrilamine.

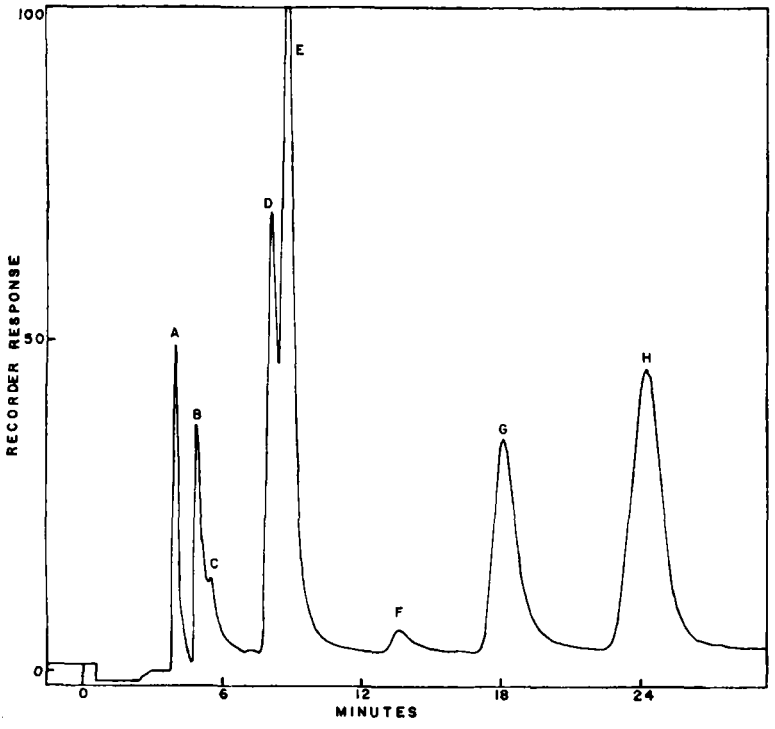

Fig. 3.—Separation using a 6-ft.—1.07% XF-1150 on 100/120 Chromosorb W-HMDS column at 174° C. Key: *A*, diphenhydramine; *B*, doxylamine; *C*, antazoline (decomposition product); *D*, methapyrilene; *E*, thenyldiamine; *F*, chlorcyclizine; *G*, thonzylamine; and *H*, pyrilamine.

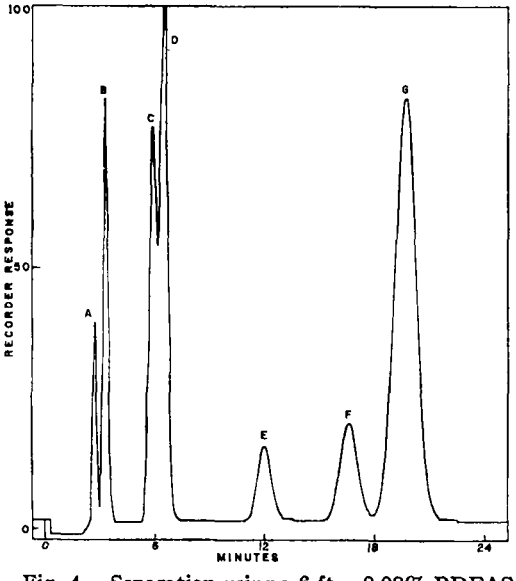

Fig. 4.—Separation using a 6-ft.—0.08% PDEAS on 120/170 glass bead column at 175° C. Key: *A*, diphenhydramine; *B*, doxylamine; *C*, methapyrilene; *D*, thenyldiamine; *E*, chlorcyclizine; *F*, thonzylamine; and *G*, promethazine, pyrilamine.

corresponds to the results reported by Fontan (11). However, during the evaluation of the last column investigated, 0.07% PDEAS on 120/170 glass beads, the first small peak was obtained, and the chromatograph was allowed to run for an extended period of time. A very strong peak was obtained with the retention time of 65.8 minutes. This indicated that the small peak obtained with the other columns was not antazoline, but an impurity or decomposition product. A retention time of 41.0 minutes was obtained by Kirk (8) using a 4 ft.-0.05% SE-30 on 60/80 mesh glass beads at 165°. No response for meclizine was obtained with the columns tested.

The first sample procedure in which aliquots of a stock solution of the antihistamine salt in water were made basic and extracted with chloroform often produced multiple peaks, indicating decomposition when injected into the chromatograph (14). The second procedure used a single weighed amount of the antihistamine salt which was dissolved in water, made basic, and extracted with chloroform. Injected samples using this method produced single peaks and showed that the decomposition experienced with the first method occurred in the stock solution of the antihistamine salt in water.

Since all column packings used in this investigation were lightly loaded, the solid support used has a

TABLE II.—MINIMUM AMOUNT NECESSARY FOR GAS CHROMATOGRAPHIC IDENTIFICATION USING A 6 FT.—0.077% PDEAS ON 120/170 GLASS-BEAD COLUMN AT 175° C.

Compd.	Amount, mcg.
Antazoline	5.4
Carbinoxamine	0.4
Chlorcyclizine	1.7
Chlorothen	0.7
Chlorpheniramine	0.4
Cyclizine	0.2
Diphenhydramine	0.2
Doxylamine	0.3
Meclizine	NR[a]
Methapyrilene	0.3
Pheniramine	0.1
Promethazine	0.2
Pyrilamine	0.8
Thenyldiamine	0.2
Thonzylamine	0.9
Tripelennamine	0.2

[a] No response.

definite bearing on the usefulness of any column packing prepared. The performances of the seven supports mentioned previously were examined under the same operating conditions. The supports that can be used for lightly loaded packings are: glass beads, Gas Chrom-P, and Chromosorb W-HMDS. The other four supports cannot be used for lightly loaded column packing since their interaction with the antihistamines causes excessive peak tailing.

The hydrogen flame detector used in conjunction with the 0.010-in. stainless capillary column would not respond to compounds with boiling points above 330°. This limitation prevented evaluation of this column for the analysis of these antihistamines.

The 100-ft. 0.065-in. copper open tubular column was coated with XF-1150 and evaluated using the above group of antihistamines. The Sr^{90} ionization detector was used with a column flow of 36 ml./minute. The retention times obtained were comparable to the 6-ft.-XF-1150 packed column, but the peak base widths were considerably wider. Because of this increase in base width, the 0.065-in. column was less efficient than the 6-ft. packed column.

A 250-ft. 0.065-in. column wound on a $1^1/_4$-in. diameter mandrel has been reported to be more efficient than a packed column (15). There are two possible reasons why efficiency was less than previously reported: (*a*) the column was shorter (100 ft.), and (*b*) the winding configuration was markedly different. The column was wound on a $1^1/_4 \times {}^1/_4$-in. bar which resulted in a definite flattening of the tube around the edge of the bar.

CONCLUSIONS

The antihistamines investigated, except for meclizine, can be separated, identified, and concentration estimated using the Carbowax 20M, PDEAS, and XF-1150 columns described. The PDEAS column is the most efficient of the three for the analysis of antihistamines.

The usefulness of the 0.010-in. capillary and the 0.065-in. open tubular columns cannot be properly evaluated until the mentioned limitations are removed.

REFERENCES

(1) Brochmann-Hanssen, E., and Svendsen, A .B., THIS JOURNAL, 51, 318(1962).
(2) Cieplinski, E. W., *Anal. Chem.*, 35, 256(1963).
(3) Parker, K. D., and Kirk, P. L., *ibid.*, 33, 428(1963).
(4) *Ibid.*, 33, 1378(1961).
(5) Brochmann-Hanssen, E., and Svendsen, A. B., THIS JOURNAL, 51, 938(1962).
(6) Parker, K. D., Fontan, C. R., and Kirk, P. L., *Anal. Chem.*, 34, 1345(1962).
(7) Fales, H. M., and Pisano, J. J., *Anal. Biochem.*, 3, 337 (1962).
(8) Parker, K. D., Fontan, C. R., and Kirk, P. L., *Anal. Chem.*, 34, 757(1962).
(9) Anders, M. W., and Mannering, G. J., *J. Chromatog.*, 7, 258(1962).
(10) Parker, K. D., Fontan, C. R., and Kirk, P. L., *Anal. Chem.*, 35, 356(1963).
(11) Fontan, C. R., Smith, W. C., and Kirk, P. L., *ibid.*, 35, 591(1963).
(12) Zubyk, W. J., and Conner, A. Z., *ibid.*, 32, 912(1960).
(13) Averill, W., "Progress in Industrial Gas Chromatography," edited by Szymanski, H. A., Plenum Press, New York, N. Y., 1961, p. 225.
(14) MacDonald, A., Jr., and Pflaum, R. T., THIS JOURNAL, 52, 816(1963).
(15) Quiram, E. R., *Anal. Chem.*, 35, 593(1963).

Castor Oil as a Vehicle for Parenteral Administration of Steroid Hormones

By C. RIFFKIN, R. HUBER, and C. H. KEYSSER

Steroid hormones may be administered parenterally in high concentrations as oil solutions. In this form they exhibit a prolonged action and reduce the number of injections required. To accommodate the demand for increasingly greater concentrations of hormones in solution, castor oil in combination with other suitable oil-miscible solvents, has been found to fulfill a need. The development of several formulations together with the results of animal testing, as well as clinical trials in humans, attest to the acceptability of this oil for the purposes intended.

FIXED OILS are included in the "United States Pharmacopeia XVI" as nonaqueous vehicles for injection and are characterized as being of vegetable origin, essentially odorless, and without suggestion of rancidity. They must also comply with certain measurable physical limits specified for the saponification, acid, and iodine values.

Received September 9, 1963, from the Pharmacology and Pharmaceutical Research & Development Sections, Squibb Institute for Medical Research, New Brunswick, N. J.
Accepted for publication November 19, 1963.
The authors are indebted to the Chemical Control Laboratories for their assistance with the assays; to Dr. N. Coy and H. Roberts of the Physical and Analytical Section, for their development of special assays; to G. Lockwood of the Pharmacology Section for the animal muscle irritation tests; and especially to Dr. E. C. Reifenstein, Jr., of the medical staff, for the information concerning the clinical trials.

After subcutaneous injection, Deanesly and Parkes (1) observed the persistence of olive oil and castor oil in animal tissue. Comparing other oils Brown, *et al.* (2), reported that sesame and corn oils were superior to cottonseed and peanut oils because they were less irritating, less antigenic, more quickly released from tissue, and possessed superior physical properties.

More recently the use of steroid hormone medication has expanded considerably. Due to limited water solubility, hormones have been administered as aqueous suspensions or solutions in oil. It has been claimed that the latter provided the slow release preferred in cyclical