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THE LIPID CONTENTS OF THE MOUSE SKIN

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ABSTRACT

The lipid contents of the mouse skin were analized by thin layer chromatography and their methyl esters of fatty acids by gas chromatography. The major component of mouse skin lipids was found to be triglyceride which consisted of 47.55% of the total lipids. Other components were: monoglycerides, free fatty acid, cholesterol ($\triangle^7$-cholesterol), diglycerides (phosphatides), cholesteryl esters and waxes, decreasing in quantity in that order. In addition to the list of lipids reported by Gunstone and Russell, 3 short chain molecules of heptanoic acid, octanoic acid and nonanoic acid were found. They came mostly from diglycerides (phosphatides) and partly from triglycerides. Monoglycerides appeared in the analysis had no saturated molecules shorter than n-hexadecanoic acid.

Since Bullough first described mitotic activity of the epidermis of the skin in the adult male mouse in 1948 (1), many related research works have been reported. Chase and Anderson described the cyclic changes of skin in the mouse (2-5). Montagna *et al.* reported that the amounts of histologically detectable acid mucopolysaccharides fluctuated during the hair growth cycle (6-7). Gerold successfully illustrated that the thickness of adipose tissue in the skin of the mouse at hair growing stage (anagen VI) was at least twice as thick as that of resting stage (telogen), but his chemical analyses showed that the fat content remained essentially unaltered during the cycles of hair growth (6). Although factors such as hormones, diet, sulfhydryl groups, enzymes (esterase), general health and genetic characteristics might influence the growth of hair (7-14), the mechanism of hair cyclic changes has not yet been understood. Recently, Ahmed and Shostak reported that the epidermis of mice deficient in essential fatty acid was nearly three times thicker than the normal one (15). Jarrett further postulated that the major function of the alkaline phosphatase activity was to hydrolyse phospholipids in epidermal cells during keratinization (16). Meanwhile, little attention has been paid to the composition of lipids in mouse skin except the sebum (17), and there is a wide gap between fatty acid metabolism and hair follicular changes. Therefore it is very desirable to reinvestigate the lipid contents of mouse skin by chemical analysis.

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MATERIALS AND METHODS

Two gm of the dorsal skin of 5 healthy male mice of NIH white strain at different hair growing stages were pooled for each sample. After plucking the hair, the skin was cut immediately into small pieces and the lipids extracted in chloroform methanol (2:1 v/v) according to the method of Folch *et al.* (18). The extracted lipids were separated by thin layer chromatography (TLC) on 'Silica gel G' (Merck) with benzene/chloroform/acetic acid (40:60:2) and petroleum ether (fraction 40°-60°)/ diethyl ether/methanol/acetic acid/acetonitrile (90:20:2:3:3) as the solvent systems. The coated plates were 0.25 and 1 mm thick, activated at 110°C for 30 min before use. The TLC fractions were made visible with chrömschwefel söner* and UV irradiation (short wave 2536 Å) after having been sprayed with 2', 7'-dichlorofluorescein (0.2%) in ethanol. The *Rf* value of each fraction was measured and located by comparing with *Rf* value of pure cholesterol marker. Then, the glyceride spots were scraped from the plates and extracted three times with 2 ml of methanol for purification. The extract fractions that had been colored with chrömschwefel söner were then analysed quantitatively using the Beckman DB spectrophotometer. The extracts that had been treated with fluorescein were evaporated to 2 ml at 50 C under reducing pressure, and were further saponified with 2 ml of 0.5 N KOH in methanol for 24 hrs at room temperature. Saponification was stopped by neutralizing with 0.5N H_2SO_4. Saponifiable fatty acids were extracted 3 times by using 5 ml petroleum ether (40-60). The petroleum ether solution was washed once with 0.5 N sodium carbonate solution. Then the free liberated fatty acids were methylated with diazomethane which was directly prepared from 'Diazald' (19). The fatty acid methyl esters were subjected to gas chromatography (The Aerograph 204B) under the conditions described in *Figs. 3-7.*

RESULTS AND DISCUSSION

I. *Thin layer chromatographical analysis*

In *Fig. 1,* using the combined method of Horsfield and Kakkainen (20-21), lipid fractions of the mouse skin including 11 components were seen. However, the resolution of diglyceride and cholesterol was not satisfactory. *Fig. 2,* using Denton's method (22) modified by adding acetonitrile to the developing solvent, shows the resolution of glycerides well; but only eight spots were detected. Being interested in glycerides, we had to adapt ourself to the second method dealing with our TLC problem. *Rf* values and relative composition (RC) (%) of four duplicates are tabulated in TABLE I.

In TABLE I, the monoglycerides, diglycerides and triglycerides were saponifiable matter, but others not, and it was clear that the major component of mouse skin lipids was triglyceride which consisted of 47.55% of the total lipids. Other components were presented in the order according to their quantity as follows: monoglycerides, free fatty acids, cholesterol, diglycerides, cholesteryl esters and waxes. However, squalene was not demonstrated. In some of our preparations which had been spotted with large amount of samples as shown in *Fig. 1,* there was another faint spot of methyl ester of fatty acid; but it might be the result of methylation of fatty acid by methanol during the extraction procedures. Even using acetone to extract the skin lipids, we had no reliable evidence to exclude this spot absolutely. Therefore, it might have existed in a very small quantity. Another possible source of cholesterol might be the mouse blood. Because of failure to perfuse the blood from the

* Chrömschwefel söner: 5 gm potassium+100 ml of 40% H_2SO_4.

TABLE I

Main lipid fractions of mouse skin

Samples		1		2		3		4		Mean	
Fractions		*Rf*	RC%	*Rf*	RC%	*Rf*	RC%	*Rf*	RC%	*Rf*	RC%
Monoglycerides		0	15.78*	0	12.30	0	12.21	0	14.81	0	13.77
Sterols and unknown	A B	0.03 0.05	4.64	0.03 0.05	3.10	0.03 0.05	4.02	0.03 0.06	4.35	0.03 0.05	4.03
Fatty acids (C_{10}-C_{20})		0.22	11.84	0.25	9.23	0.27	9.90	0.23	11.11	0.24	10.52
Cholesterol (△7-cholesterol)		0.32	8.55	0.32	10.76	0.35	10.55	0.32	11.72	0.32	10.39
Diglycerides and phospholipids (Lecithin etc.)		0.45	6.57	0.46	10.46	0.47	9.24	0.45	8.02	0.45	8.57
Triglycerides and vitamin A		0.83	47.69	0.85	49.23	0.89	48.84	0.85	44.44	0.85	47.55
Cholesteryl esters and waxes, diol esters		0.94	4.93	0.93	4.92	0.97	5.28	0.95	5.55	0.94	5.17

* Percentage of light absorbance at 320 u minus control (background of thin layer)

samples completely, our data on the relative composition of cholesterol might be slightly higher than the actual composition of the skin. Other fractions like fat soluble vitamins might exist, but their detailed analysis is beyond the scope of this report.

II. *Gas chromatographical analysis*

In spite the suggestion of Stoffel (23) that the use of alkali and diazomethane for methylation of fatty acids might lead to isomerization or pyrazoline formation, we found that Herman's methods were very good. *Figs. 4-7* were records of the methyl esters of fatty acids of skin lipid mixtures, monoglycerides, diglycerides and triglycerides respectively. Their peak areas measured by triangulation were tabulated in TABLE II. In *Fig. 4,* the peaks were mainly heptanoic, octanoic, nonanoic, decanoic, dodecanoic, tridecanoic, tetradecanoic, hexadecanoic, hexadecenoic, octadecanoic, △-octadecanoic, △-octadecadienoic and △-octadecatrienoic. In *Fig. 5,* the peaks were n-hexadecanoic, △-hexadecenoic, octadecenoic. In *Fig. 6,* the peaks were n-nonanoic, n-decanoic and n-undecanoic. In *Fig. 7,* most of the peaks looked alike to those of *Fig. 4,* but different in relative composition. The results may also convince us to believe that most of the fatty acids of skin lipids come from triglycerides. During the experiment, although the chromatogram was run for 40 minutes, arachidonic molecules that had been illustrated by Wheatly (17) in the sebum of the mouse were not detected. This might illustrate the different properties of depot lipids of the skin from hair lipids (24). The result of long chain molecules of skin lipids were also in accord to the data of Gunstone and Russell (25) that the hexadecanoic acid, octadecenoic acid and those unsaturated octadecenoic acids from monoene to triene were the principle contents of depot fat of the mouse (*Fig. 4*). The shorter chain molecules such as heptanoic, octanoic and nonanoic that have not been recorded before came mostly from diglycerides and partly from triglycerides, while the monoglycerides were not demonstrated. We could not make sure whether they were present in the skin in

TABLE II

Relative percentages of fatty acids of mouse skin lipids saponified from T.L.C fractions

Peaks	Chemical names	Standard designation	Retention volume	Lipid mixture	T. L. C. Fractions		
					Monogly-cerides	Digly-cerides	Trigly-cerides
1	n−Heptanoic	7:0	0.13*	1.64		5.72	1.84
2	n−Octanoic	8:0	0.22	2.34		3.25	0.07
3	n−Nonanoic	9:0	0.30	3.08		17.88	8.59
4	n−Decanoic	10:0	0.40	2.32		12.44	5.18
5	n−Undecanoic	11:0	0.48	3.39		12.79	5.01
6	n−Dodecanoic	12:0	0.59	3.69		5.72	2.98
7	n−Tridecanoic	13:0	0.69	3.61		3.64	
8	n−Tetradecanoic	14:0	0.79	3.94			2.70
9	n−Pentadecanoic	15:0	0.88	2.87			2.64
10	n−Hexadecanoic	16:0	1.00	4.49	8.10		4.23
11	$\triangle^{9}$−Hexadecanoic	16:1	1.02*	5.20	9.81		7.36
12	n−Heptadecanoic	17:0	1.11	4.02	9.09		
13	n−Octadecanoic	18:0	1.26	4.06	14.85		5.52
14	△−Octadecanoic	18:1	1.33*	5.88	15.81		7.52
15	△−Octadecadianoic	18:2	1.47*	4.59	9.71		5.74
16	△−Octadecatrianoic	18:3	1.61*	2.46	10.38		5.04
17	n−Nonodecanoic	19:0	1.48				
18	n−Eicosanoic	20:0	1.83	2.10			
19	n−Heneicosanoic & △−Heneicosanoic	21:0 21:1-3	2.30	1.57	11.68	6.11	5.85
20	Undefined	>22:0	>2.97				15.22
	Unknowns**			38.75	10.57	32.45	14.51

* Obtained by comparing with known published data(17).

**Including other undefined isomers, branched chains of fatty acids, and other compounds.

large quantities, because side reactions might have happened during the slow saponification procedures. This is worthy of further quantitative study.

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LITERATURE CITED

1. BULLOUGH, W. S. 1948. Mitotic activity in the adult male mouse. *Proc. Roy. Soc.* **B135:** 212-233.
2. CHASE H. B., W. MONTAGNA and J. D. MALONE 1953. Changes in the skin in relation to the hair growth cycle. *Anat. Rec.* **116:** 75-82.
3. CHASE H. B. 1954. Growth of the hair. *Physiol. Rev.* **34:** 113-126.
4. ANDERSON E. 1953. Cyclic changes in skin of the mouse. *Acta Path. et Microbial. Scandi.* **32:** 157-164.

5. CHASE H. B. and G. J. EATON. 1959. The growth of hair follicles in waves. *Ann. N. Y. Acad. Sc.* **83:** 365-368.
6. GEROLD, N. B. and W. MONTAGNA 1956. Fat in the skin of the mouse during cycles of hair growth. *J. Invest. Dermatol.* **26:** 229-232.
7. MONTAGNA, W., H. B. CHASE J. D. MALONE and H. P. MELORAGNO. 1958. Cyclic changes in polysaccharides of the papilla of the hair follicle. *Quart. J. Micro. Sc.* **93:** 241-245.
8. EISEN A. Z., W. MONTAGNA and H. B. CHASE. 1953. Sulfhydryl groups in the skin of the mouse and guinea pigs. *J. Nat. Cancer Inst.* **14:** 341-353.
9. SHIPMAN M., H. B. CHASE and W. MONTAGNA. 1955. Glycogen in the skin of the mouse during cycles of hair growth. *Proc. Soc. Exper. Biol. & Med.* **88:** 449-451.
10. BAKER B. L. 1951. Relationships of the adrenal, thyroid and pituitary glands to the growth of hair. *Ann. N. Y. Acad. Sc.* **53:** 690-707.
11. DAVIS B. K. 1963. Quantitative morphological studies upon the influence of the endocrine system on the growth of hair in white mice. *Acta Endocrinologica* **44:** supplement 85.
12. LOWONTHAL L. A. and W. MONTAGNA 1955. Effects of caloric restriction on skin and hair growth in mice. *J. Invest. Dermatol.* **24:** 429-433.
13. MONTAGNA W. and V. R. FORMISAM. 1963. Esterase activity in the skin of mammals. *J. Anat. London* **89:** 425-429.
14. FRIEDMAN-KIEN A. E., C. J. DAWE and E. J. VAN SCOTT 1964. Hair growth cycle in subcutaneous implants of skin. *J. Invest. Dermatol.* **43:** 445-450.
15. AHMED N. N. and S. SHOSTAK 1965. Mitotic activity in the skin of mice deficient in essential fatty acids. *Nature* **207:** 1395.
16. JARRETT, A. R. I. C. SPEARMAN P. A. RILEY and A. K. CANE. 1965. The distribution of epidermal phospholipids and their relation to the alkaline phosphatase activity of the granular layer. *J. Invest. Dermatol.* **44:** 311-319.
17. FOLCH J., I. ASCOLI, M. LEE and J. A. MEATH. 1951. Preparation of lipid extract from brain tissue. *J. Biol. Chem.* **191:** 833-841.
18. WHEATLY, V. R. and A. T. JAMES 1957. Studies of sebum. VII. The composition of the sebum of some common rodents. *Biochem. J.* **65:** 36-42.
19. HERMAN, S. and J. L. GELLERMAN. 1960. Esterification of fatty acids with diazomethane on a small scale. *Analytical Chem.* **32:** 1412-1414.
20. HORSFIELD G. I. and H. J. YARDLEY 1965. Sclerema neonatorum. *J. Invest. Dermatol.* **44:** 326-332.
21. KARKKAINEN J., T. NIKKARI S. RUPONEN and E. HAAHTI 1965. Lipids of vernix caseosa. *J. Invest. Dermatol.* **44:** 333-338.
22. DENTON, R. M. and P. J. RANDLE. 1965. Hormonal control of lipid concentration in rat heart and gastrocnemius. *Nature* **208:** 488.
23. STOFFEL W., F. CHU and E. H. AHRENS, JR. 1959. Analysis of long chain fatty acids by gas liquid chromatography. *Analytical Chem.* **31:** 307-308.
24. SMITH Q. T., M. F. RAMON and G. GRANDE 1965. Fatty acids of hair lipids of germ free and germ exposed rats. *J. Invest. Dermatol.* **44:** 348-350.
25. GUNSTONE, F. D. and W. C. RUSSELL. 1957. Animal fats. VII. Component acids of fats from mouse, porcupine, and rabbit. *J. Sc. Agri.* **8:** 283-286.

LEGEND OF FIGURES

Fig. 1. Fractionation of an extract of skin lipids of mice on a thin layer of silica gel (1 mm). Bands were visulized using the method of chrömschwefel söner, (400 u1).

Fig. 2. Thin layer chromatography of mouse skin lipids of 6 subjects (0.25 mm). Spots were visualized by using 2', 7'-dichlorofluorescein and UV irradiation, (3 ug each spot).

Fig. 3. Gas chromatography of the standard methylesters of fatty acid on polyethylene glycol succinate at programming temperature, 6 C/min from 100 C to 175 C.

Conditions for chromatography: Column specification, 8 ft x 1/8 inch coil-shaped copper tube; solid support, chromosorb W acid-washed, stationary phase, (mesh 60/80); 15% polyethylene glycol succinate; carrier gas, nitrogen 40 ml/min; detector, hydrogen flame ionization, hydrogen rate 30 ml/min; time of analysis, 40 minutes; recorder, speedmax, 0.1 mv full scale; temperature of injection block, 250 C; temperature of detector, 240 C; pressure, inlet 5 atm, outlet 1 atm; sample size, 2λ; petroleum ether; attenuation, 4.

Fig. 4. Gas chromatography of the lipid mixture of mouse skin on polyethylene glycol succinate at programming temperature. Conditions as for *Fig. 3* except sample size 1.5λ; attenuation, 2.

Fig. 5. Gas chromatography of the monoglycerides of mouse skin. Conditions as for *Fig. 3* except sample size, 4λ; attenuation, 2.

Fig. 6. Gas chromatography of the diglycerides of mouse skin. Conditions as for *Fig. 3* except sample size, 4λ; attenuation, 2.

Fig. 7. Gas chromatography of the triglycerides of mouse skin. Conditions as for *Fig. 3* except sample size, 4λ; attenuation, 2.

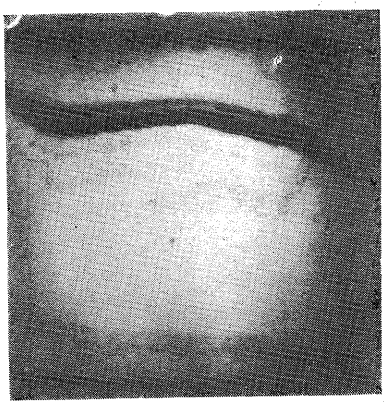

Fig. 1

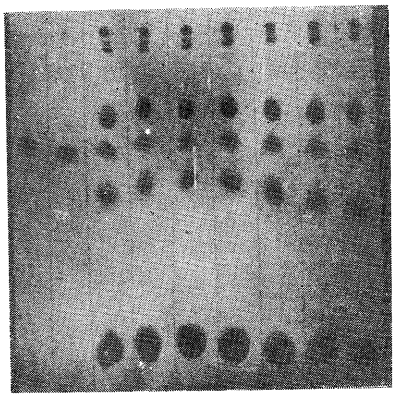

Fig. 2

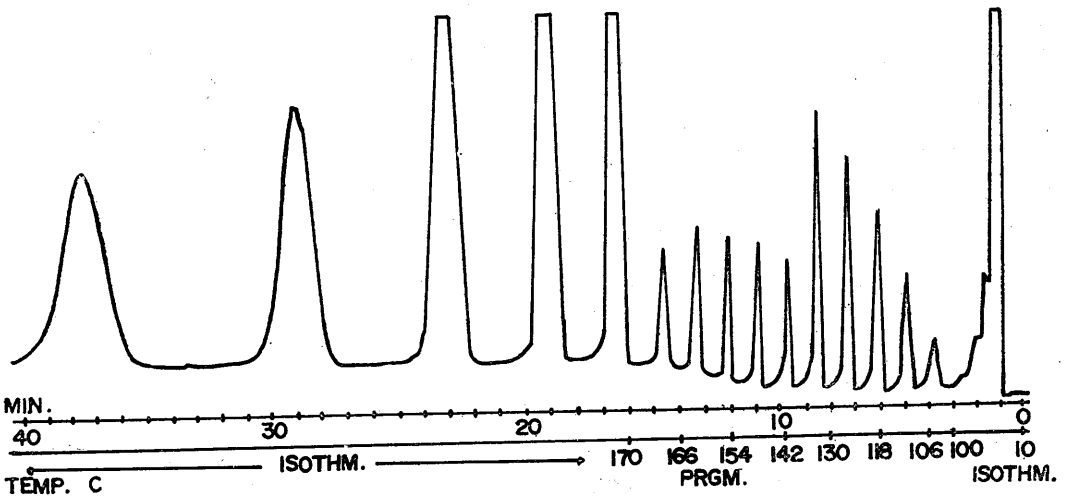

Fig. 3

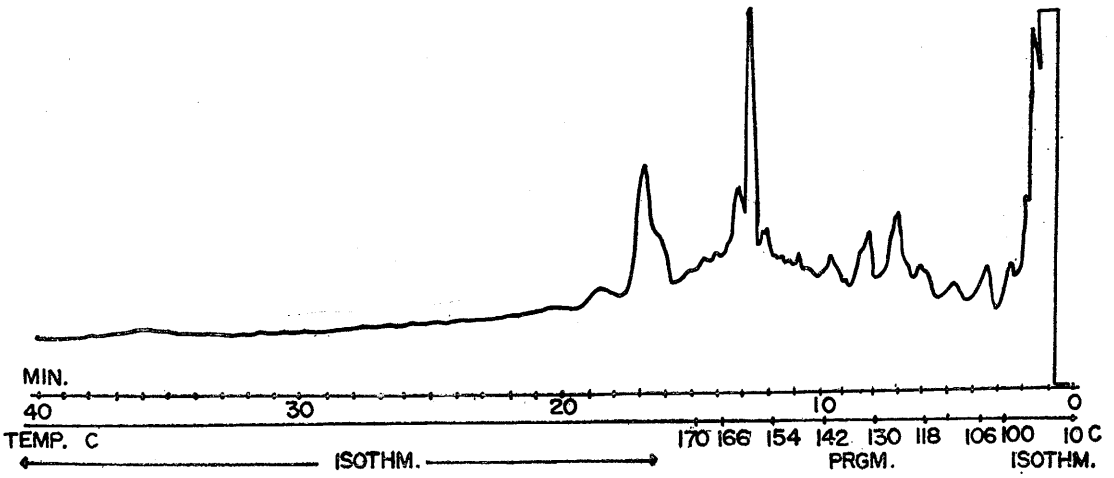

Fig. 4

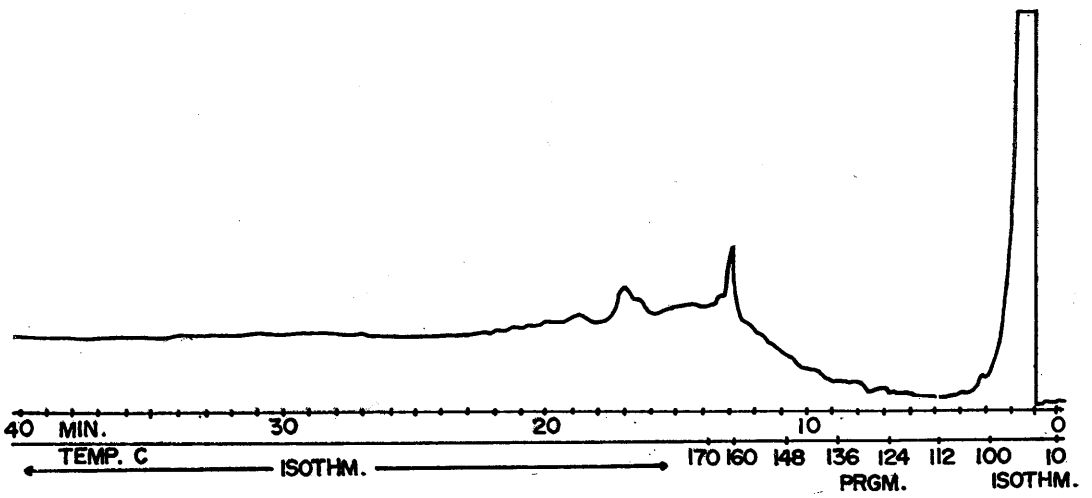
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Fig. 5

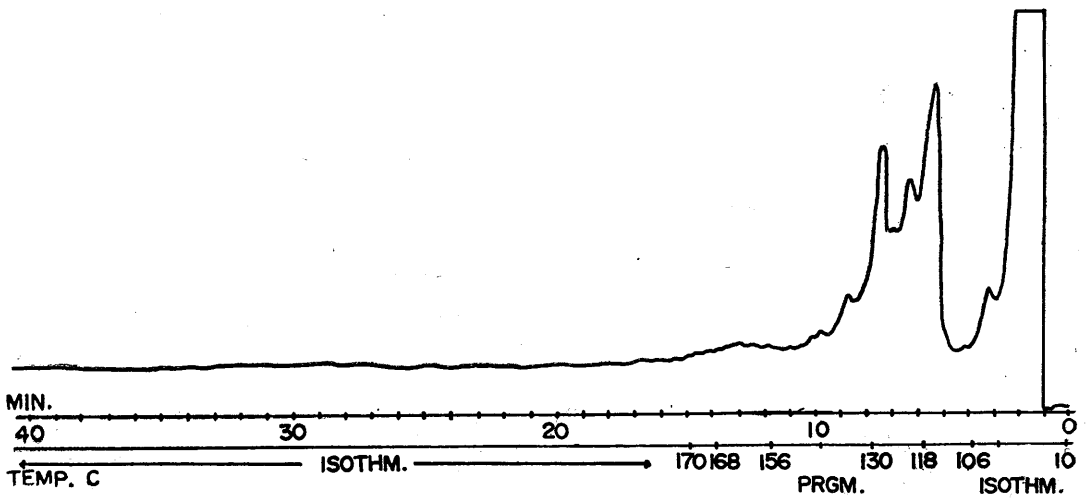
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Fig. 6

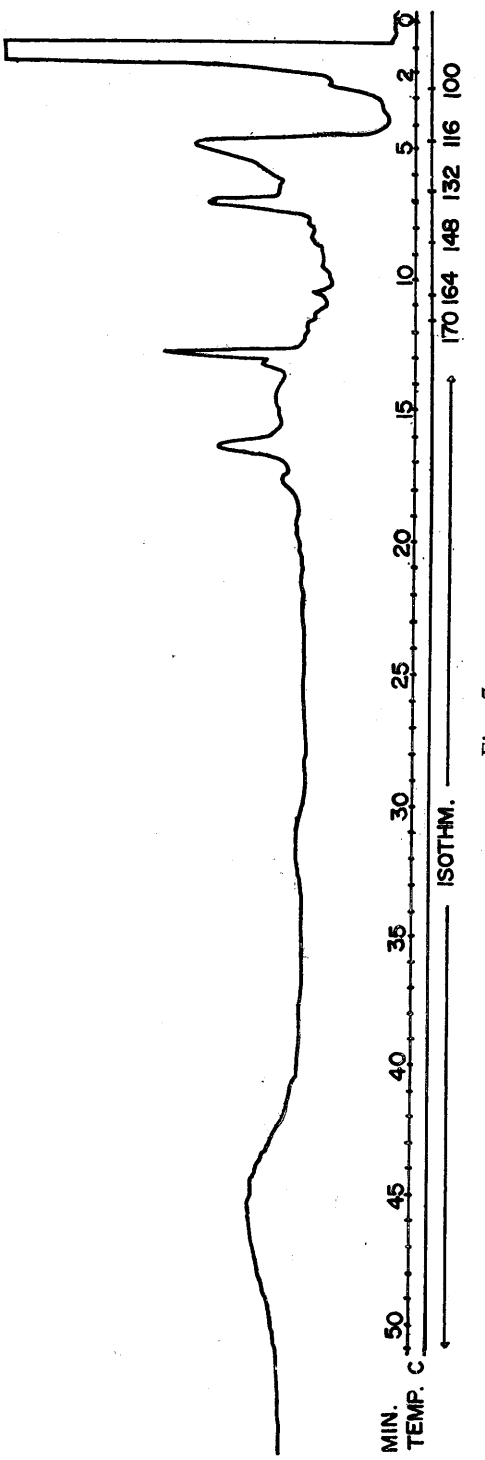
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Fig. 7