FRACTIONATION OF HYDROCARBONS OF HUMAN HAIR LIPIDS BY CHROMATOGRAPHIC AND THERMAL DIFFUSION METHODS

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INTRODUCTION

The occurrence of paraffinic hydrocarbons as bona fide constituents of human sebum has been questioned by some workers. Thus, NICOLAIDES AND ROTHMAN[1] reported the presence of substantial amounts of saturated hydrocarbons in hair lipids but in a later study[2], these components were regarded as contaminants since with subsequent products, the hydrocarbon level fell considerably. Lipid extracted from the forearm with acetone was shown to contain 7.5 % paraffins[3,4], an amount comparable to those reported for hair lipids by HAAHTI[5] and GERSHBEIN AND KROTOSZYNSKI[6]. The latter workers fractionated the mixtures in a preliminary fashion into waxes (about 2 %) and liquid hydrocarbons. The level of the liquid components fell when the lipids were processed from scalp wipings of subjects with alopecia and crew cuts. However, in such cases, the nature of the "sebum" would be quite altered in view of the composition of epidermal lipids which have been shown to be quite distinct as compared to hair as a source[7]. It was later suggested that the paraffins of forearm lipids might be external skin contaminants[8]. The authenticity of the hydrocarbons as sebum components has been discussed by HENSEKE AND SCHIEFFER[9] and the widespread occurrence of hydrocarbons is reviewed by ORÓ, NOONER AND WIKSTROM[10].

In the present study, two large pools of lipids extracted from the hair of adult full-headed white and Negro men were saponified and the unsaponifiable portion (UNS) analyzed, greater emphasis being directed to the hydrocarbon components. Among others, column chromatographic and thermal diffusion techniques were explored toward the isolation of enriched fractions for further analysis by gas chromatography.

The criterion of liquid thermal diffusion has been reviewed and greatly extended by JONES AND MILBERGER[11]. By this method, based on CLUSIUS AND DICKEL type of diffusion, it is possible to bring about a separation of liquid mixtures which normally resist various conventional methods. In practice, a column is used which consists of two concentric tubes, the outer one being heated electrically and the inner or "cold" tube, cooled by circulating cold water (adjusted so that the temperature rise is under 10°). In the 5 ft. column described by the above authors, the slit width, namely, the average distance between the walls was 0.0115 in. and the mean slit diameter, 0.63 in.; total volume: 22.5 ml. Withdrawal ports located at 6 in. intervals

along the column allowed for the removal of 10 individual cuts. After equilibration over a protracted period of time, the fluid was drained first from the upper port and then successively to the bottom of the column. The efficacy of fractionation by thermal diffusion does not depend on molecular weights as many isomers can be separated successfully. The molecular shape or configuration is the most important determining factor. Also, the relative concentration of compounds figures prominently. Thus, good resolution of a benzene–octadecane mixture was achieved only when benzene occurred in large excess.

EXPERIMENTAL

Lipid samples and reagents

Unless otherwise stated, the techniques for the extraction of lipids from hair by petroleum ether (b.p. 30–60°C) and determination of constants were identical with those presented earlier[6,12,13]. The reagents were of high grade or A.R. purity. All solvents were redistillled and pertinent collecting vessels in the processing of hair and lipids were degreased with ligroin and ethyl ether. Hair cuttings were pooled individually from white and Negro full-headed adult males who used no hair dressings, whatsoever and shampooed their hair with bar soap every 5–7 days. For the pooled lipids, WF-7-L and CF-7-L, in the order stated, the yields, iodine numbers and acid numbers were 3.6%, 67.9 and 99.4, and 6.4%, 66.4 and 99.3, respectively.

FRACTIONATION OF HAIR LIPID POOL CF-7-L AND HYDROCARBON ISOLATION

Saponification

A total of 937 g hair lipids was processed in two portions, 1.4 l 20 % sodium hydroxide in 95 % ethanol being used; duration of refluxing: 24 h. The cooled reaction mixtures were each taken up in 16 l water and extracted six times with ether (over-all volume: 20 l); the extract was washed with water until free of alkali. Removal of ether after drying the solution over anhydrous sodium sulfate yielded UNS. The aqueous layer and washings were acidified with sulfuric acid and steam distilled until essentially free of volatile acid as determined by titration against alkali. The steam distillate was made weakly alkaline with KOH and on extraction with ether yielded no significant amount of product when concentrated. The residue after steam distillation was chilled, the mixture of fatty acids taken up in ether, washed with water, dried and concentrated. In the saponification of one portion of the hair lipids (462 g), UNS amounted to 156 g (33.6 %) and the mixed fatty acids, to 271 g (58.7 %). Steam distillation yielded 0.98 g of volatile acids.

Chromatography of UNS over activated alumina

In the manner described earlier[6], UNS in amounts of 282 g was dissolved in 28 l petroleum ether and the solution passed through a column containing 9.0 kg activated alumina (Alcoa F-20). Elution of the column with 9 l each of the solvents (1) petroleum ether, (2) petroleum ether + 5 vol. % chloroform, (3) petroleum ether + 10 vol. % chloroform, (4) chloroform and (5) absolute methanol yielded Fractions I–V, inclusive, on removal of the respective solvents. The yields, properties and pertinent constants are presented in Table I.

TABLE I

CHROMATOGRAPHY OF UNS FROM HAIR LIPID POOL CF-7-L OVER ALUMINA[a]

Fraction (eluent)	*Recovery*		*Properties of fraction*		
	Wt. (g)	*% in UNS*	*Iodine No.*	n_D^{20}	*Appearance*
I (petroleum ether)	113.7	40.3	92		White plastic mixture
II (petroleum ether–5 % $CHCl_3$)	11.6	4.1	289	1.5006	Light, clear oil of sharp odor
III (petroleum ether–10 % $CHCl_3$)	2.9	1.0	150	1.5300	Very thick yellow oil with characteristic mild odor
IV ($CHCl_3$)	11.5	4.1	145	1.5022	Heavy orange oil of sharp odor
V (absolute methanol)	137.2	48.6	62		Orange solid and oil mixture

[a] A total of 282 g UNS was chromatographed with a recovery of 98.1 %.

Isolation of "oils" from Fractions I–IV

Frac. I-Oil. Acetone was used for the separation of solid hydrocarbons. It has proved effective as shown earlier[6] in the resolution of Fraction I into (a) wax, insoluble in cold or hot acetone, (b) "triacontane", insoluble in the cold and (c) Frac. I-Oil, a fluid miscible with the solvent. The yields as based on Fraction I were: "triacontane", 6.1 %; wax, 9.4 %; Frac. I-Oil, 83.9 %; recovery: 99.4 %.

Frac. II-Oil. When 7.22 g Fraction II was heated with 50 ml acetone then chilled for 1 h at 0°C, a solid separated (142 mg; 1.9 %) which had a consistency similar to the wax of Fraction I. The oil weighed 6.77 g (95.7 %).

Frac. III-Oil. As per the last experiment, 1.48 g Fraction III on treatment with 15 ml acetone gave rise to 1.25 g Frac. III-Oil (84.5 %; n_D^{20} 1.5331) and 124 mg wax (8.4 %). Due to some losses, the recovery was 92.9 %.

Frac. IV-Oil. Of a total of 10.56 g Fraction IV, only 147 mg of a yellow solid separated on treatment with 50 ml hot acetone followed by chilling. The residual heavy oil was dark orange in color n_D^{20}; 1.5095.

Silica gel chromatography of Frac. I-Oil

With the view of separating hydrocarbons of hair lipid pools according to chemical type, elution chromatography on silica gel was attempted with numerous synthetic mixtures. Due to excessive heat evolution, recourse was made to water-cooled columns. In all cases, the activated silica gel was Davison's 100–200 mesh and elution with five successive media was found to be most feasible as illustrated in the following runs.

Expt. 1-59. A solution of 1.5 g oil in 50 ml petroleum ether was passed over a column containing 37 g silica gel. The eluting solvents comprised the following, each in amount of 60 ml and in the order given: (1) petroleum ether, (2) benzene, (3) ethyl ether, (4) acetone and (5) anhydrous methanol. A total of 21 cuts was collected of which the first four (petroleum ether-elution) after removal of solvent, displayed n_D^{20} values of 1.4670, 1.4722, 1.4722 and 1.4755, respectively, and were saturated. The components in the benzene cuts were highly unsaturated and showed a blue fluorescence; the refractive indices were higher (for example, for Cut 8, a light yellow oil,

the n_D^{20} was 1.4990). The residues from the ethyl ether-eluted cuts were yellow and exhibited a greenish fluorescence. The movement of this material along the column was accompanied by heat evolution. Methanol gave rise to a material containing traces of aromatic components.

Expt. 1-69. The above experiment was repeated with a larger sample of oil (10.06 g; 2 % solution in petroleum ether); weight of silica gel: 292 g. The volume of each eluting solvent was 600 ml. Twenty-eight 100 ml cuts were accumulated (Fig. 1).

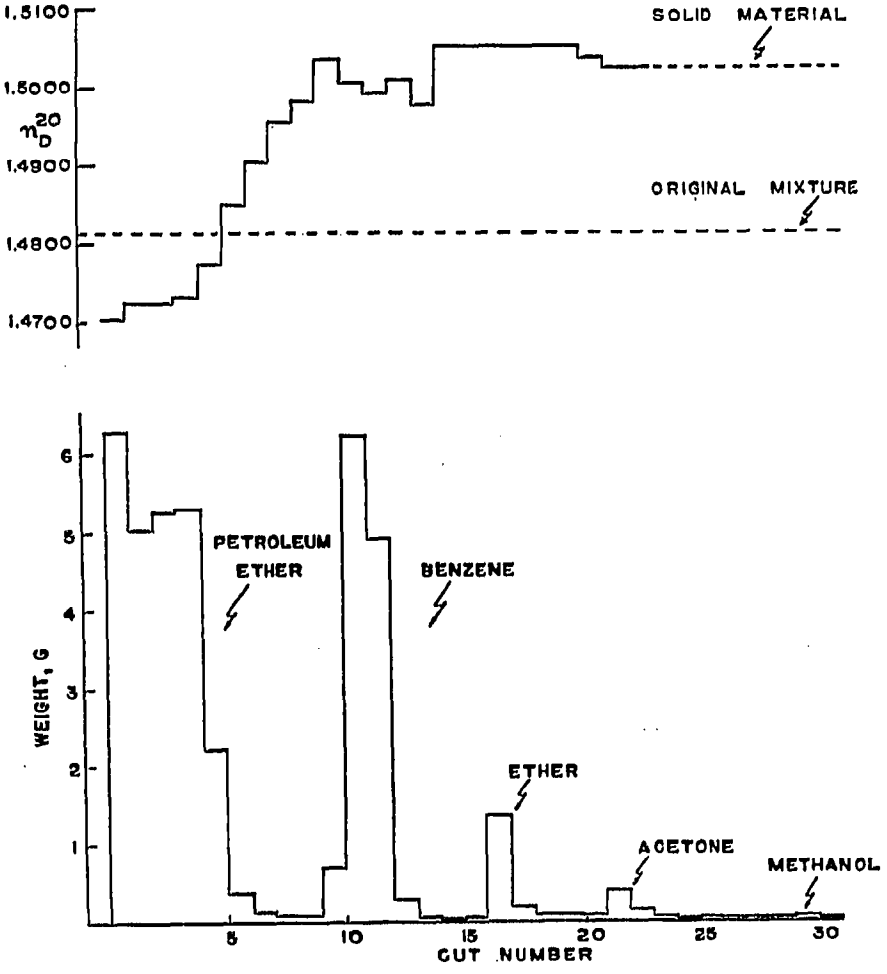

Fig. 1. Weights and indices of refraction of cuts removed by silica gel chromatography of Fract. I-Oil of Pool CF-7-L (Expt. 40-1; 40.0 g).

The d_{20}^{20} of Cuts 1, 2 and 4 (petroleum ether-elution) and No. 9 (benzene) were 0.8518, 0.8507, 0.8681 and 0.8894, respectively. The last fraction was a heavy oil with an iodine number of 263. The petroleum ether-eluted cuts were saturated and free of aromatic hydrocarbons. The recovery amounted to 9.83 g or 98.0 %.

Expt. 40-1. In this run, the oil in amount of 40.0 g was dissolved in 2 l petroleum ether and the mixture passed over 1197 g of silica gel in a 5 × 122 cm column (length of packing: 1 m). By use of 2.4 l of each of the eluting media, thirty-one 400 ml cuts were collected under gravity, the distribution and indices of refraction of the components of which are shown in Fig. 1; the over-all recovery was 98.5 %. Cuts 1–9 were heavy, water-clear, odorless saturated oils. The n_D^{20} of Cuts 1 (6.29 g), 2 and 3

(10.23 g) and 4 (5.26 g) were 1.4704, 1.4724, 1.4724 and 1.4732, respectively, the values increasing up to 1.4980 for No. 9. Cuts 10–16 (n_D^{20} 1.503–1.505) were heavy yellow oils with definite sharp odor; the iodine number of No. 12 was 293. The unsaturated fractions were stored under nitrogen at 0°C due to their instability. Thus, a portion of Cut 10 underwent some polymerization after 5 days at 25°C. The n_D^{20} of each of Cuts 15–20, inclusive, was 1.5050; No. 17, a very heavy clear yellow oil, had an iodine number of 210. Cut 20, a very tacky yellow fluid of mild odor contained traces of aromatic components. Cuts 21–23 were similar in consistency, the iodine number of Cut 22 being 117. Solid occurred in Cuts 23–31, inclusive.

Rechromatography of petroleum ether-eluted hydrocarbons. Cuts 1–4 of Expt. 1-69 and Cuts 1–5 of Expt. 40-1 were combined, the mixture (29.47 g; 2 % solution in petroleum ether) chromatographed over 900 g silica gel and the column eluted with 500 ml portions of the solvents. A total of 24 cuts was collected as shown in Fig. 2. For Cuts 1–3, inclusive, making up 9.8, 6.7 and 5.8 % of the total, the n_D^{20} values were 1.4680, 1.4719 and 1.4724 in the order stated and for each of Cuts 4–13 (63 %), 1.4730.

Cut 1 (2.73 g; Fig. 2) in petroleum ether solution was rechromatographed over silica gel (120 g) and the column eluted solely with petroleum ether (150 ml); 8–15 ml fractions were obtained, the recovery being 97 %. The n_D^{20} was 1.4664 for No. 1, a range of 1.4680–1.4686 for Cuts 2–5 (69 %), inclusive and 1.4742 for No. 6 (5.7 %).

Vacuum distillation of liquid saturated hydrocarbons. Cuts 2–13, inclusive

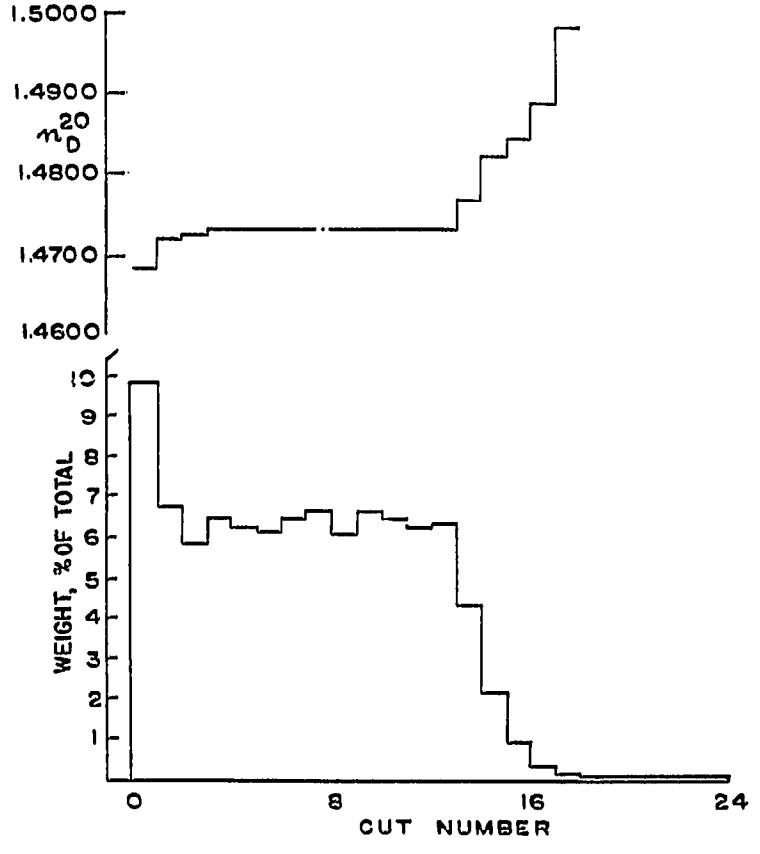

Fig. 2. Yields and indices of refraction of petroleum ether-eluted cuts from rechromatography of saturated hydrocarbons (Cuts 1–5, Fig. 1 and 1–4, inclusive of Expt. 1-69) over silica gel.

(Fig. 2), were combined and 20.0 ml (n_D^{20} 1.4723; d_{20}^{20} 0.8619) distilled in a 5 mm × 90 cm Podbielniak spinning band column at 1 mm pressure. The thruput and product rates were 15 drops/min (or 15 ml/h) and 0.5 ml/h, respectively; reflux ratio: 30/1. The distillation was stopped when the pot went dry; duration: 22 h. The cumulative recovery, bottoms and residues (removed with benzene) amounted to 14.9, 2.7 and 1.7 ml in the order given, corresponding to a total recovery of 96.5 %. The pertinent curves are shown in Fig. 3 and representative analytical data appear in Table II.

Silica gel chromatography of "oils" from Fractions II, III and IV

Frac. II-Oil (Expt. 1-30). A total of 6.10 g Frac. II-Oil was chromatographed in 1 % petroleum ether solution in a water-cooled column charged with 205 g silica gel; 41 cuts were removed, the recovery being 98 % (Fig. 4). Cuts 1–15, inclusive, making up 3.8 % of the total, were combined and comprised a yellowish oil of rather sharp unpleasant odor. Cuts 17–23 (80.7 %) were pale, heavy clear oils of mild odor; the n_D^{20} of each of Cuts 17–21 was 1.5018 and of No. 25 (10.1 %), 1.5020.

Frac. III-Oil (Expt. 1-35B). Similar to the above, Frac. III-Oil, a clear orange oil of sharp odor and displaying blue fluorescence, was submitted to chromatography over silica gel (1202 mg oil; 1 % solution in petroleum ether; 36 g gel; volume of each eluting medium: 60 ml). Thirty-four cuts were obtained and recovery was

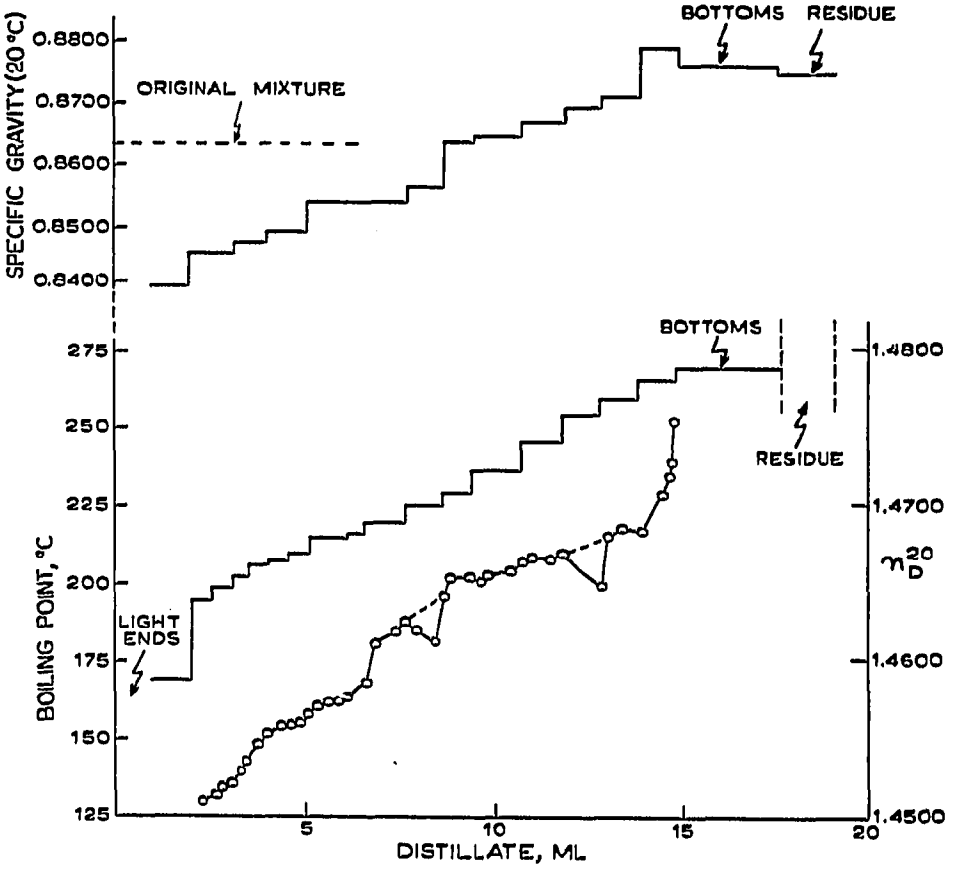

Fig. 3. Boiling points, indices of refraction and densities of the respective cuts from distillation of the saturated hydrocarbon mixture (Cuts 2–13, inclusive of Fig. 2; 20.0 ml) at 1 mm pressure. Because of fluctuations in pressure with a few cuts, the dotted lines show the more probable location of points at 1 mm.

TABLE II

CONSTANTS OF REPRESENTATIVE CUTS FROM DISTILLATION OF CF-7-L SATURATED HYDROCARBONS[a]

Cut No.	*Volume (ml)*	*B.p. (1 mm)*[b]	n_D^{20}	d_{20}^{20}	*Analysis*	
—					*% C*	*% H*
2	0.49	132.2	1.4639	0.8443		
3	0.52	136.2	1.4647	0.8459	85.57	13.95
6[c]	0.52	154.6	1.4664	0.8526	86.45	13.93
7	0.50	157.8	1.4669	0.8526		
9	0.50	163.9	1.4679	0.8531		
11	1.06	188.6	1.4689	0.8553	86.23	13.94
13	1.00	200.1	1.4708	0.8624		
15	1.06	209.2	1.4740	0.8657	85.90	13.36

[a] Sample comprised Cuts 2–13, inclusive, Fig. 2, in amount of 20.0 ml. Distillation curve appears in Fig. 3.
[b] Final temperature reading for the cut.
[c] Molecular weight: 311 (cryoscopic; benzene as solvent).

essentially quantitative (Fig. 5). Petroleum ether-eluted cuts (1–14, inclusive) amounted to 4.3 % of the total and those from benzene elution (15–21, inclusive), occurred in greatest yield (91 %). The n_D^{20} of Cuts 15, 16 and 17 were 1.5368, 1.5368 and 1.5386, respectively; the iodine number of Cut 16 was 183. Of the ether-eluted fractions (22–26, inclusive; 6.5 %), No. 22 was most prominent (5.4 %; n_D^{20} 1.5052).

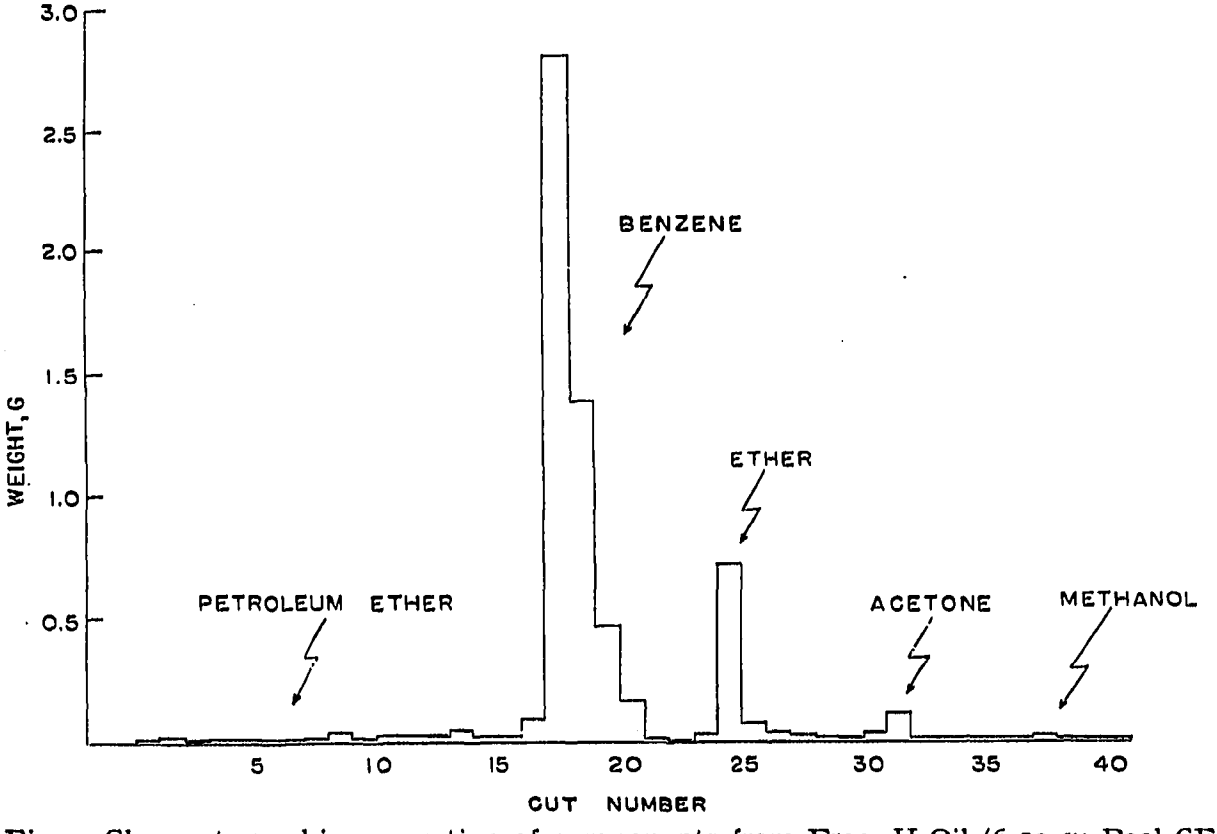

Fig. 4. Chromatographic separation of components from Frac. II-Oil (6.10 g; Pool CF-7-L) over silica gel.

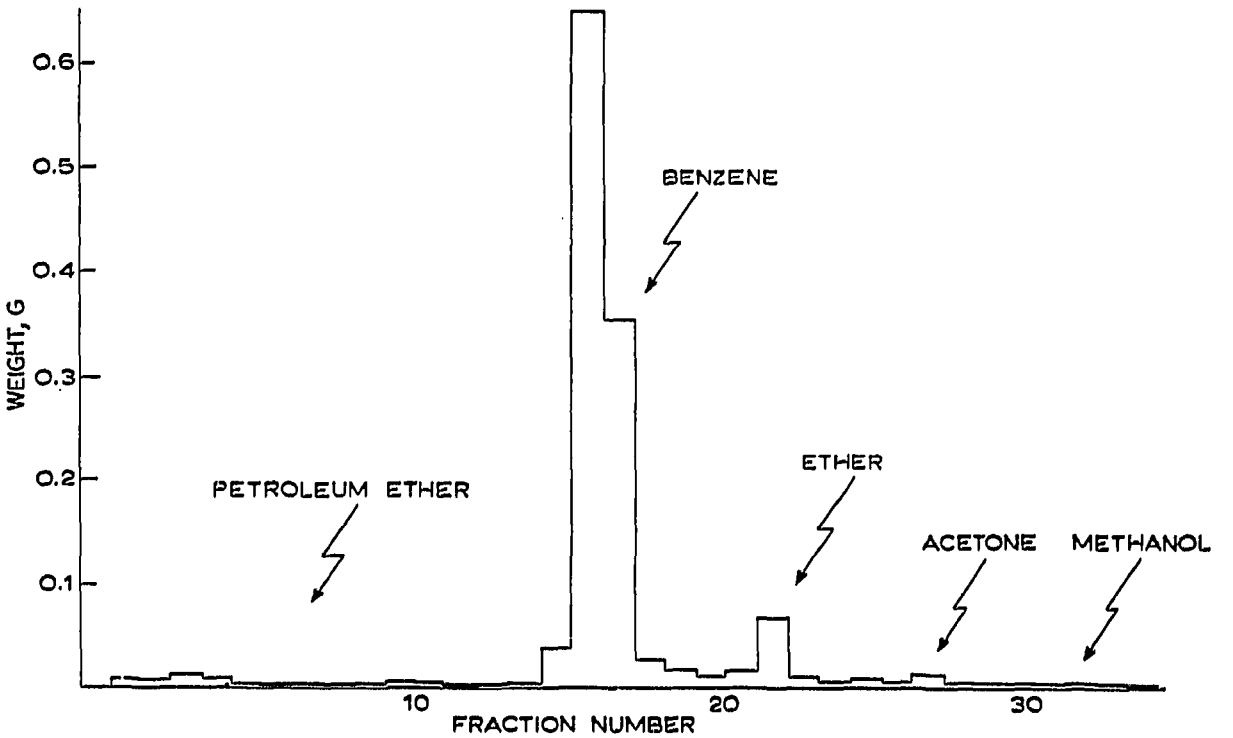

Fig. 5. Silica gel chromatographic resolution of Frac. III-Oil from Pool CF-7-L (1202 mg).

Frac. IV-Oil (Expt. 4-37). A solution of 6.59 g Frac. IV-Oil, a highly fluorescent fluid, in 330 ml petroleum ether was chromatographed over 220 g gel by the general procedure, the volume of each solvent being 400 ml, except that elution with acetone was omitted; recovery: 96 %. Petroleum ether removed a colorless oil which made up only 1 % of the starting material. The iodine number of the most prominent fraction (No. 12; 62.6 %), a very heavy, orange-red oil of pleasant odor, was 228; aromatic components were absent, although traces seemed to occur in Cuts 8–11, inclusive. The iodine number of Cut 8, an orange oil of mild odor, was 93. The distribution of fractions is given in Fig. 6. In contrast to oils of Fractions I–III, inclusive, infrared and ultraviolet absorption spectra showed the presence of ketonic moieties possibly with α,β-unsaturation.

FRACTIONATION OF HAIR LIPID POOL WF-9-L

Saponification and isolation of Frac. I-Oil

Hair lipids pooled from adult full-headed white males in amount of 558 g was saponified with 20 % NaOH in ethanol (1.6 ml/g lipid); duration of heating: 24 h. The resulting mixture was taken up in 11 l of water and extracted portionwise with ethyl ether (25 l); yield of UNS: 177 g (31.6 %; iodine number: 115).

When a solution of 168 g UNS in 16.8 l petroleum ether was passed over 2.3 kg alumina and the column eluted with 500 ml each of petroleum ether as such and containing 5 % and 10 % chloroform, 100 % chloroform and finally methanol, the yields of Fractions I, II and III were 61.7 g (36.7 %), 6.3 g (3.7 %) and 0.60 g (0.35 %), respectively, the iodine numbers being 145, 348 and 91, in the order stated. Fraction

IV made up 7.9 g or 4.7 % and the remainder comprised the alcoholic mixture, Fraction V.

By treatment of 51.7 g Fraction I with 1 l boiling acetone, the following were isolated: wax, 2.0 g (3.5 %); "triacontane", 2.4 g (4.2 %) and Frac. I-Oil, 25.7 g (88.4 %; n_D^{20} 1.4832).

Chromatography of Frac. I-Oil over silica gel

Expt. A3-20. A 2 % solution of Frac. I-Oil (25.7 g) in petroleum ether was introduced over 393 g silica gel and elution carried out with 800 ml portions of the respective media; 16–250 ml cuts were collected with a recovery of 98 %. The n_D^{20} for Cut 1 (3.26 g; 12.7 %) was 1.4710 and for the remaining saturated hydrocarbons of Cuts 2–5, inclusive (10.29 g; 40.0 %), 1.4723–1.4732. Cut 10 (10.15 g) was a clear yellow oil of pleasant odor, n_D^{20} 1.4973 and iodine number, 367.

Expt. A5-40. Similar to the last experiment, 20.4 g of the oil was chromatographed (400 g gel; volume of eluting solvents: 600 ml) and 32 cuts removed. The distribution of components simulated the previous run.

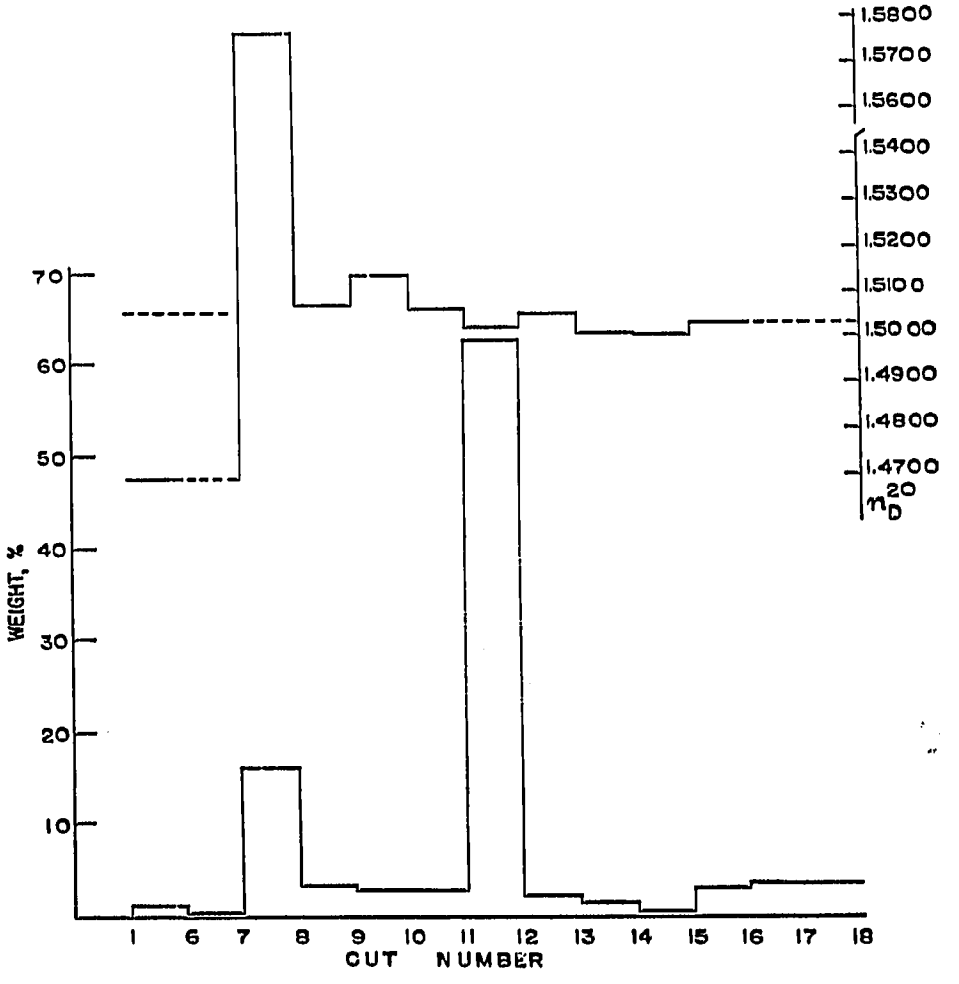

Fig. 6. Weights and indices of refraction of cuts from chromatography of Frac. IV-Oil 6.59 g Pool CF-7-L) over silica gel and elution of the column with the usual five media.

Thermal diffusion analysis of saturated hydrocarbons

Cuts 1–5 of Expt. A3-20 and Cuts 1–9, inclusive of Expt. A5-40, each group representing 53 % of Frac. I-Oil were combined and 21.1 g (27 ml) submitted to thermal diffusion analysis in a column with a capacity of 30 ml and equipped with 20 removal ports. It will be noted that this column was somewhat larger than the one described by JONES AND MILBERGER[11]. As insufficient liquid was available, complete filling of the column was not possible and accordingly, no fluid could be drained above the fifth uppermost port. The column was operated at a temperature difference of 145°F, the hot and cold walls being maintained at 205°F and 60°F (tap water), respectively; duration: 336 h. The pertinent data, including representative elementary analyses and average molecular weights obtained ebullioscopically with benzene as solvent, appear in Table III. The progressive changes in refractive indices and densities can be noted from Fig. 7. The recovery was 89 % on a weight basis and is somewhat low because of incomplete drainage of the lower ports containing the very viscous fluids. Thus, Cut 15 had the consistency of a very thick syrup.

TABLE III

PROPERTIES OF CUTS FROM THERMAL DIFFUSION ANALYSIS OF SEBUM WF-9-L SATURATED HYDROCARBONS[a, b]

Cut No.	*Wt. (g)*	*% of total*	n_D^{20}	d_{20}^{20}	*% C*	*% H*	*Molecular weight*[c]
1	0.65	3.0	1.4489[d]	0.8175[d]	85.14	14.70	309
2	1.37	6.4	1.4511	0.8112			
3	1.67	7.8	1.4548	0.8118			
4	0.75	3.5	1.4577	0.8151			
5	1.40	6.6	1.4588	0.8167			
6	1.16	5.4	1.4610	0.8289			
7	1.19	5.6	1.4648	0.8366	86.30	14.08	329
8	1.27	6.0	1.4669	0.8366			
9	1.36	6.3	1.4690	0.8410			
10	1.20	5.6	1.4739	0.8558			
11	1.21	5.6	1.4776	0.8663			
12	1.61	7.5	1.4816	0.8850	86.55	13.52	376
13	1.37	6.4	1.4913	0.9112			
14	1.50	7.0	1.4964	0.9234			
15	1.53	7.1	1.4987	0.9401	87.47	12.68	412
Original mixture			1.4718	0.8605			371

[a] Mixture from combination of Cuts 1–5 of Expt. A3-20 and Cuts 1–9 of Expt. A5-40; 21.1 g. The data are plotted in Fig. 7.
[b] Recovery: 89% (weight basis).
[c] Ebullioscopic method (benzene as solvent).
[d] Determined at 25°C since the fraction was solid at 20°C.

Distribution of squalene in the hydrocarbon fractions

Squalene was analyzed by the gas chromatographic procedure of O'NEILL AND GERSHBEIN[13]. It occurred solely in Fractions I, II and III from chromatography of UNS over alumina. The values in terms of the three fractions were in good agreement with those obtained on direct analysis of UNS. Squalene was especially prominent in the benzene-eluted cuts from the oils of Fractions I–III, inclusive. In this regard,

TABLE IV

COMPARISON OF SILICA GEL CHROMATOGRAPHIC DATA FOR FRACTION I-OILS FROM SEBUM POOLS WF-9-L AND CF-7-L

Eluting medium	*Distribution, %*[a]	
	WF-9-L	*CF-7-L*
Petroleum ether	53.3	59.9
Benzene	42.9	31.9
Ethyl ether	2.0	4.6
Acetone	0.9	1.5
Methanol	0.3	0.6

[a] All percentages are based on Frac. I-Oil.

the distribution of the various cuts from silica gel chromatography of UNS of CF-7-L and WF-9-L is summarized in Table IV. The squalene contents based on the initial hair lipids were 4.6 and 5.7 % for CF-7-L and WF-9-L, respectively.

GAS CHROMATOGRAPHIC ANALYSIS OF SATURATED HYDROCARBONS

Liquid hydrocarbon samples were submitted to temperature programmed gas chromatography in a Barber-Colman Model 5000 instrument with hydrogen detector

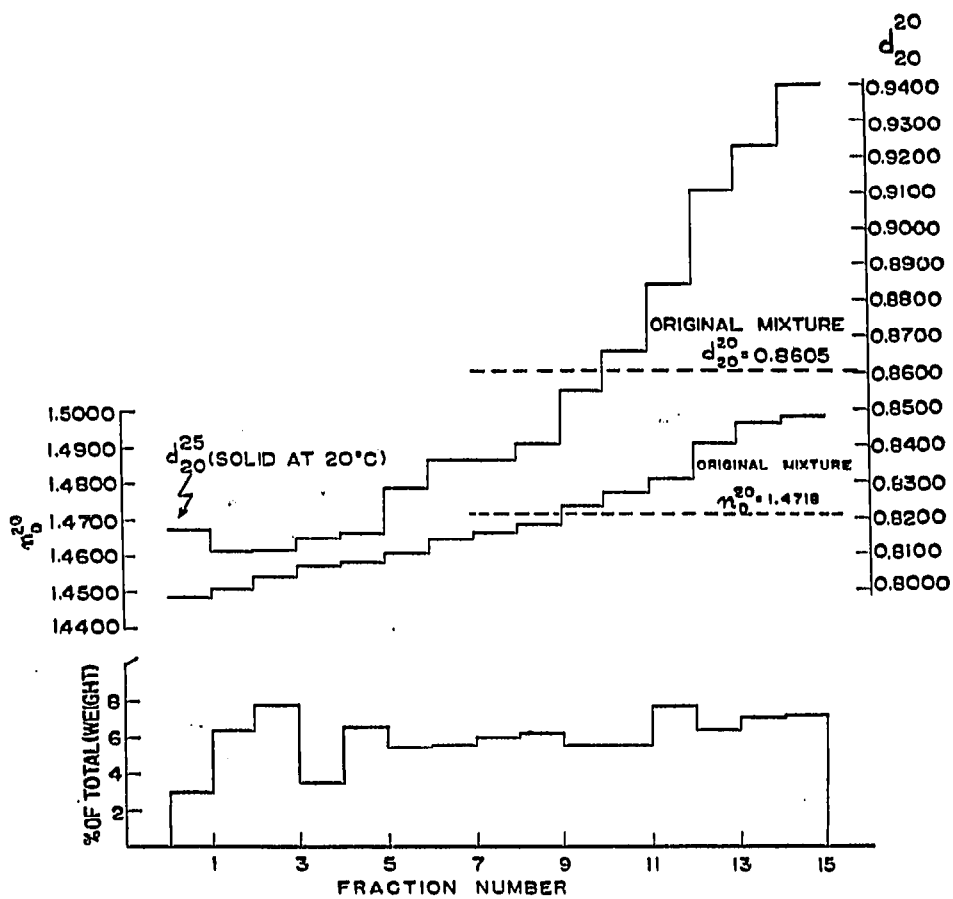

Fig. 7. Weight percentages, indices of refraction and densities of cuts from thermal diffusion analysis of Pool WF-9-L saturated hydrocarbons (petroleum ether eluate; 21.1 g).

to 275°C and the wax mixtures, to 325°C. The rate of heating was 2°C/min. The U-shaped glass column measured 72 × 0.6 in. O.D. and was packed with 3% SE-30 on 80–100 mesh Gas Chrom P. The detector temperature was 320° or 350°C depending on the sample. The carrier gas was He at 12 lb. pressure. The column was preconditioned at 350°C with He for 16 h. The samples were injected in ethereal solution and the peaks identified by comparison with the elution times. Normal saturated hydrocarbon mixtures up to C_{29} were employed as standards in addition to a few higher homologs made available to the authors by several German laboratories. The liquid saturated hydrocarbon mixtures derived from both hair lipid pools yielded similar peaks and an analysis of the WF-9-L product together with cuts from the thermal diffusion is presented in Table V. The qualitative distribution of hydrocarbons in representative cuts from the distillation of hydrocarbons from Pool CF-7-L (Fig. 3) is shown in Table VI. The solid paraffinic hydrocarbons ranged up to C_{44} and were all normal in structure (Table VII) and except for very small differences in amounts of the lower

TABLE V

RELATIVE PERCENTAGE COMPOSITION OF SEBUM WF-9-L SATURATED HYDROCARBON MIXTURE AND SOME CUTS FROM THERMAL DIFFUSION[a, b]

Relative carbon No.	*Initial mixture*	*Percentage*		
		Cut 1	*Cut 2*	*Cut 3*
15	0.5	1.2	0.8	0.3
15.2		0.5		0.6
15.4	1.1	1.2	1.3	
15.6		0.5	0.9	1.2
15.8	1.1	1.4		0.9
15.9		0.7		
16	1.6	2.3	2.6	1.5
16.2	1.6	0.9	0.9	0.9
16.5	2.2	4.4	4.5	2.2
17	3.9	1.6	5.5	2.8
17.3	1.6			
17.5	1.1	5.1	0.9	1.2
17.8		1.2		
18	3.8	5.1	5.6	3.7
18.5	1.1	0.9	1.1	1.5
19	6.0	8.3	8.3	5.6
19.5	1.1	1.4	1.9	3.4
20	6.0	8.6	7.9	4.9
20.5	1.6	2.1	1.9	1.9
20.8	1.1			1.3
21	2.2	9.0	9.4	5.6
21.3				1.9
21.5	6.0	1.2	0.9	
21.8	2.7	1.9	1.5	2.5
22	4.4	8.8	9.2	5.9
22.5	3.8	1.2	0.9	2.8
22.8		1.6	1.5	2.8
23	4.4	6.3	5.8	6.2
23.5	1.6	0.9	1.1	2.5
23.8	4.4	1.4	1.7	3.1
24	4.3	5.1	4.5	5.8
24.3	2.2	1.2		
24.5	1.6	0.7	1.5	1.5

(continued on p. 443)

TABLE V (*continued*)

Relative carbon No.	*Initial mixture*	*Percentage*		
		Cut 1	*Cut 2*	*Cut 3*
24.8			1.1	0.9
25	4.4	3.0	3.4	4.6
25.3		0.9		
25.5	2.7	0.7	1.1	1.2
25.8			0.9	1.2
26	2.2	1.6	1.1	1.5
26.3				1.5
26.5	4.9	0.9	0.8	1.2
26.8	1.1	0.7	0.6	0.9
27	2.7	0.9	0.7	1.9
27.5	0.5	0.9	0.8	0.6
27.8		0.5	0.8	
28	1.6	0.5	0.8	1.2
28.5	1.1	0.7	0.4	0.6
29	1.6	0.7	0.8	1.5
29.5		0.2	0.4	0.6
29.8			0.4	
30	1.1	0.5	0.6	0.9
31		0.5	0.6	1.0
32		0.5	0.8	1.0
33			0.4	0.9

[a] Temperature programmed gas chromatographic separation over SE-30. For data on cuts from the thermal diffusion, see Table II and Fig. 3. The values are area percentages.

[b] Cuts 4–15, inclusive, showed the presence of components up to C_{33} (with Cut 6: to C_{34} trace). The initial starting relative carbon No. and the total number of peaks in the chromatogram for the remaining thermal diffusion cuts were: (4) C_{15}, 43; (5) C_{15}, 45; (6) $C_{15.8}$ trace, 33; (7) C_{16} trace, 32; (8) C_{17} trace, 28; (9) $C_{17.5}$, 30; (10) C_{18}, 22; (11) C_{20}, 18; (12) C_{20}, 18; (13) C_{20}, 21; (14) $C_{20.5}$, 18 and (15) $C_{20.5}$ trace, 18, respectively.

homologs, the wax and "triacontane" products fractionated by acetone as described above were quite similar. Typical chromatograms for the liquid hydrocarbon mixture and "triacontane" derived from Pool WF-9-L are illustrated in Figs. 8 and 9, respectively.

DISCUSSION

The saponification of hair lipids and chromatography of UNS over alumina followed by elution with petroleum ether alone and in mixture with 5 % and 10 % chloroform, 100 % chloroform and finally methanol, led to Fractions I–V, inclusive. Hydrocarbons occurred in Fractions I, II and III and were essentially absent in Fraction IV; Fraction V contained sterol and alcohols, exclusively[6]. The separation of hydrocarbons into solid and liquid types was afforded by treatment of the fractions with acetone, the portion insoluble in hot solvent being a waxy mixture melting over a range of 51–55°C and the "triacontane" or hot acetone-soluble solid of m.p. 62–63°C. Normal or straight chain paraffin hydrocarbons occurred exclusively in both (C_{20}–C_{44}; Table VII). The respective residual oils contained squalene and saturated hydrocarbons. The latter as well as the solid paraffins were markedly lower in Fractions II and III, squalene and possibly other unsaturated members predominating.

TABLE VI

DISTRIBUTION OF HYDROCARBONS IN CUTS FROM DISTILLATION OF SEBUM CF-7-L SATURATED HYDROCARBONS[a, b]

Relative carbon No.	*Distillation cut No.*									
	1	*2*	*3*	*4*	*8*	*10*	*12*	*15*	*18*	*Still residue*
15	+									
15.4	+	+	+	T						
15.6				+						
16	+	+	+	+						
16.2	+									
16.5	+	+	T	+						
17	+	+	+	+						
17.3	+									
17.5	+	+		+						
17.8		+	+	T						
18	+	+	+	+						
18.5	+	+	+	+						
19	+	+	+	+						
19.5	+	+	+							
20	+	+	+	+	+	T				
20.5		+	+	+	+					
20.8	T		+							
21		+	+	+	+	+				
21.5		+		+	+	+	+			
21.8			+							
22		T	T	+	+	+	+			
22.5				+	+	+	+			
22.8				+	+	+	+			
23				+	+	+	+			
23.5					+	+	+			
23.8					+	+	+			
24					+	+	+			
24.3					+	+				
24.5					+	+	+			
24.8						+				
25						+	+	+		
25.5						+	+	+		
25.8							+			
26						T	+	+		
26.5							+	+		
26.8								+		
27								+	+	
27.5								+	+	
27.8								+		
28								+	+	T
28.5								+	+	+
28.8								+		
29								T	+	+
29.5									+	
30									+	+
30.5										+
31									+	+
32									+	+
33										+
34										T

[a] Cuts as per Fig. 3.
[b] A (+) sign denotes presence of the component; T, trace.

TABLE VII

GAS CHROMATOGRAPHIC SEPARATION OF WAXY PARAFFINIC HYDROCARBONS ("TRIACONTANE") FROM FRACTION I-OIL OF POOL WF-9-L

Relative carbon No.	%
20	T*
21	T
22	0.7
23	1.5
24	4.0
25	7.6
26	9.9
27	11.7
28	10.4
29	10.7
30	7.6
31	11.2
32	5.1
33	4.8
34	2.3
35	2.2
36	1.5
37	1.5
38	1.6
39	1.3
40	1.0
41	1.0
42	0.8
43	1.0
44	0.8

* T = trace.

Further resolution of hydrocarbons was achieved by column chromatography of the fraction oils in petroleum ether over silica gel. Saturated hydrocarbons occurred in the petroleum ether-eluted cuts and the olefinic types were removed by benzene, ethyl ether, acetone and methanol; squalene was especially high in the portions eluted with benzene. The saturated hydrocarbons comprised 60 % of Frac. I-Oil and about 4 % each of Fractions II and III from Pool CF-7-L.

It should be pointed out that although squalene was high in the benzene-eluted cuts, other unsaturated and as yet unidentified components might also be present. Thus, considering the constants for squalene (n_D^{20} 1.4965 and d_{20}^{20} 0.8538), the prominent olefinic hydrocarbon cuts, especially those from Fraction III (Fig. 5), displayed indices of refraction ranging from 1.537 to 1.539 and iodine numbers far under the anticipated value. The presence of very small amounts of aromatic hydrocarbons might also be considered in view of the fluorescence described for a number of cuts in the previous section. In this conjunction, the resolution of the highly fluorescent Fraction IV into a variety of viscous and colored unsaturated oils but of lower iodine number and very high indices of refraction (Fig. 6), has been affected by silica gel chromatography and these as well as the above hydrocarbon cuts are currently undergoing further study.

A comparison of components based on constants and pertinent fractionation procedures, indicates a close similarity between the hair lipid pools from adult male

J. Chromatog., 27 (1967) 431–449

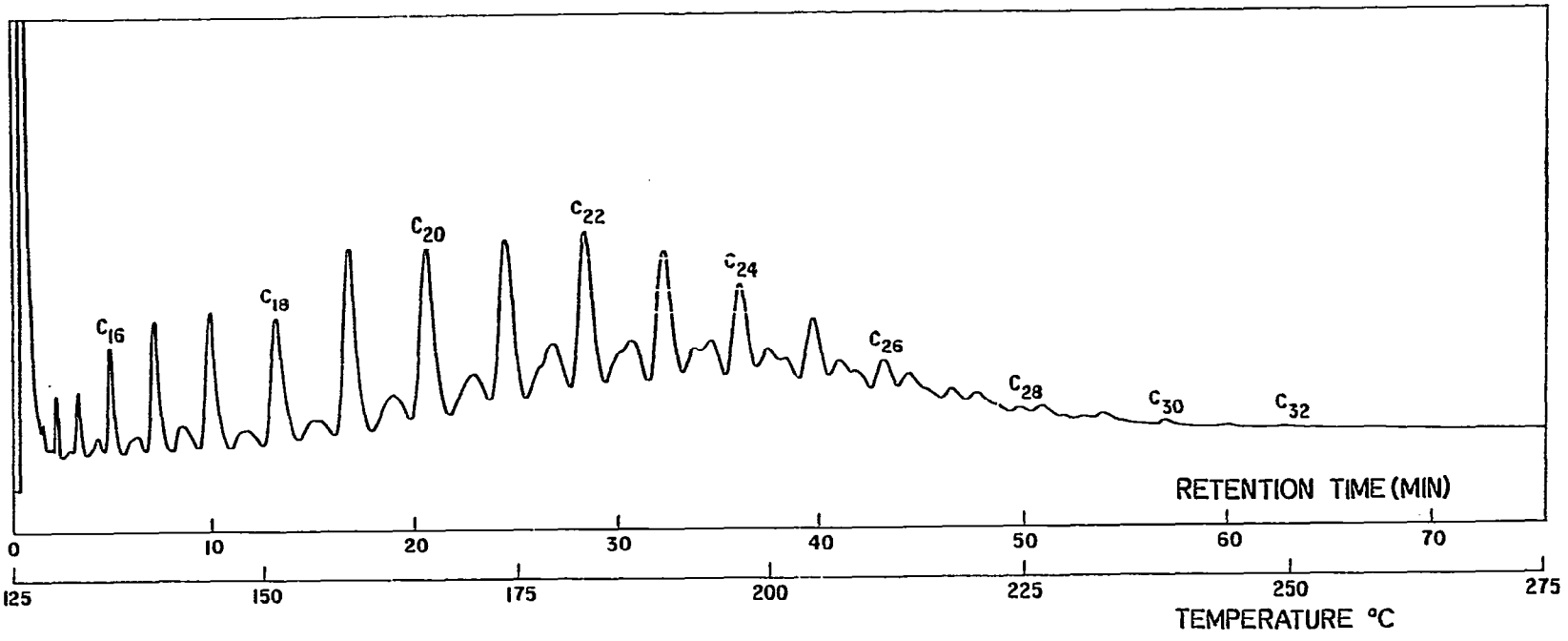

Fig. 8. Analysis of liquid saturated hydrocarbon mixture from Pool WF-9-L by temperaure programmed gas chromatography.

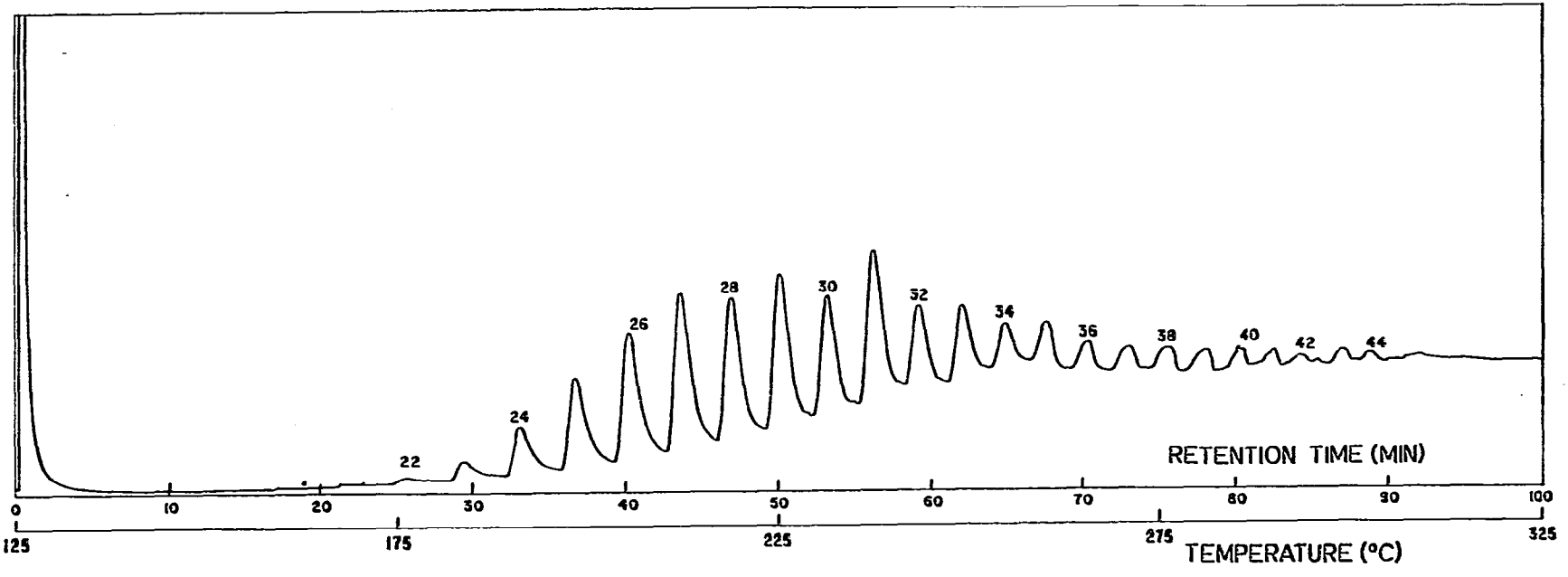

Fig. 9. Gas chromatographic separation of solid hydrocarbons ("triacontane") from treatment of Frac. I-Oil of Pool WF-9-L with acetone.

white and Negro subjects, WF-9-L and CF-7-L, respectively, except possibly for a lower squalene content for the last product (4.6 %; for WF-9-L: 5.7 % in terms of the initial lipids). The decrease in squalene is also reflected in the diminished percentage of the benzene-eluted components from CF-7-L and which was also noted with the oils from Fractions II and III. The saturated hydrocarbon mixtures from petroleum ether-elution of the gel column also ranged higher with CF-7-L and which might be the case to a minor extent with the cuts obtained with ethyl ether, acetone and methanol (Table IV).

Of a number of silica gel chromatographic runs with the oils of Fraction I from Pools CF-7-L and WF-9-L, the petroleum ether-eluted or saturated hydrocarbons displayed n_D^{20} values in the range of 1.4722–1.4732 and d_{20}^{20} of 0.852–0.868 for most of the cuts. When the cuts from the two CF-7-L runs were combined, rechromatographed over the gel and the column eluted solely with petroleum ether, the n_D^{20} was essentially 1.4730 throughout except for 7 % with values up to 1.493 and for the first cut making up 10 % of the total with an index of 1.4680. When the latter was also rechromatographed over silica gel, the indices of the fractions comprising 70 % of the mixture ranged from 1.4680 to 1.4686. From the elevated indices of refraction and specific gravity of the mixed saturated hydrocarbons, the level of naphthenic components appeared to be quite significant, a point later amplified by thermal diffusion studies and temperature programmed gas chromatography. The liquid saturated hydrocarbons of Pool WF-9-L displayed about 40 peaks indicative of C_{15} to C_{30}. The 16 normal paraffins in this range were represented and made up about 40 % of the entirety, the remaining members being branched and cyclic in nature (Table V). A chromatogram depicting *n*-paraffins to C_{30} in hair lipids has been advanced by HAAHTI[5].

Toward the isolation of various discrete fractions preliminary to characterization, the saturated hydrocarbon mixture was distilled in an efficient column at 1 mm pressure. Many cuts were collected but few clearly delineated plateaus occurred though progressive changes in index of refraction and specific gravity were noted (Fig. 3; Table II). Gas chromatographic analysis of these cuts showed over 12 components in each (Table VI). An enrichment of cyclic and acyclic hydrocarbons was affected by thermal diffusion, the product from gel chromatography of the oil from Fraction I of Pool WF-9-L constituting the starting material (Fig. 7; Table III). By means of the densities, indices of refraction and the average molecular weights, some characterization of the mixtures was afforded by the *n-d-M* method[14]. The latter has been used in the evaluation of carbon distribution and ring content of olefin-free petroleum fractions or oils boiling above the gasoline range. Determination of carbon distribution in the present case, namely, in the absence of aromatic components, involved evaluation of the percentage of carbon atoms in naphthenic nuclei (% C_N) and in paraffins (% C_P). The data depicting the distribution of carbon, C_P and C_N, for the cuts are presented in Fig. 10. By this method, the mean naphthenic ring number per molecule (R_N) was calculated for each as shown in Fig. 11. As would be expected, the % C_P decreased progressively with cuts withdrawn toward the bottom ports of the thermal diffusion column and concurrently, the % C_N together with the naphthenic ring number increased. The complexity of the hydrocarbon mixture can be noted from the distribution of R_N, the highest mean number of naphthene rings per molecule being about 5.5 and from the gas chromatographic analyses (Table V). The paraffinic

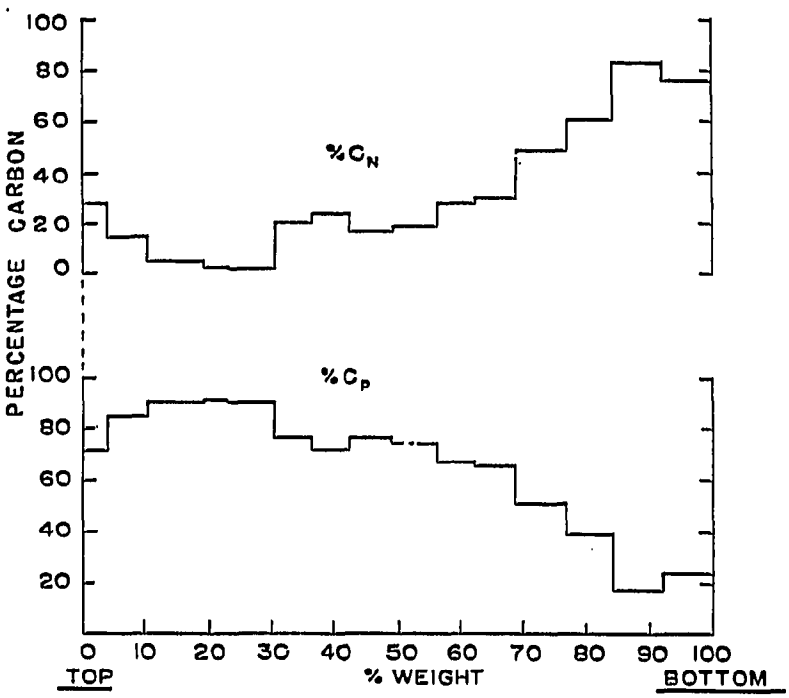

Fig. 10. Distribution of carbon (C_P and C_N) in the cuts resulting from thermal diffusion (Fig. 7) as determined by the *n-d-M* Method.

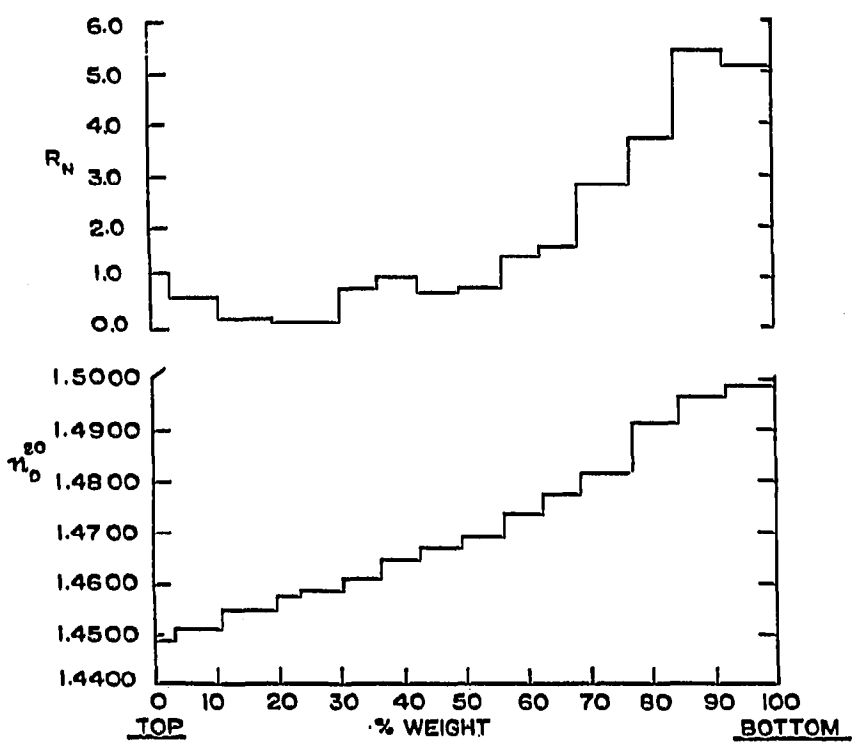

Fig. 11. Mean naphthenic ring number (R_N) of the cuts from thermal diffusion calculated by the *n-d-M* method.

hydrocarbon content paralleled the C_P by the above calculations. Resolution of about 11 components which apparently overlapped in the initial chromatogram could be discerned in the analyses of the cuts as was also the case with the higher *n*-paraffins, C_{31} to C_{34}, inclusive.

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SUMMARY

Hair lipids from adult white and Negro men were individually pooled, the two batches saponified and the unsaponifiable portions in petroleum ether chromatographed over alumina. The column on elution with petroleum ether as such and containing 5 and 10% chloroform, yielded Fractions I–III, inclusive, on removal of the solvents; 100% chloroform eluted another portion, Fraction IV, which in contrast to the latter three, was essentially free of hydrocarbons. Waxy components of C_{20}–C_{44}, all normal hydrocarbons, were obtained from Fractions I, II and III by treatment with acetone and ranged highest in Fraction I. The residual fluid (Frac. I-Oil) was chromatographed in petroleum ether solution over silica gel and contained olefinic hydrocarbons in addition to paraffinic and naphthenic components. The corresponding oils from Fractions II and III were high in unsaturated hydrocarbons, notably squalene. Good enrichment of the saturated hydrocarbons was accomplished by use of thermal diffusion. The distribution of carbon (C_P and C_N) and the mean naphthenic number per molecule (R_N) for cuts obtained by this criterion were calculated by the *n-d-M* method; the highest R_N was in the range of 5.5. Temperature programmed gas chromatography showed the presence of C_{15}–C_{34} hydrocarbons in the initial liquid mixture and derived cuts, all of the normal paraffins in this range being represented as summarized in Table V.

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