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PARAFFIN OIL PNEUMONIA

ANALYSIS OF SATURATED HYDROCARBONS IN DIFFERENT HUMAN TISSUES

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SUMMARY

Temperature-programmed gas chromatographic analysis on columns packed with Apiezon L as stationary phase is shown to be the best method for the qualitative and quantitative analysis of simple and complex hydrocarbon mixtures when compared with all the other applicable techniques (thin-layer chromatography, column chromatography, ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry) described in this paper. Using the method in a patient with mineral oil pneumonia it could be demonstrated that he expectorated a maximum of 79.5 mg liquid paraffin daily and also transported equally complex saturated hydrocarbons in a concentration of 1.3 mg% in plasma and of 1.6 mg% in the cellular blood components. In an additional experiment the direct determination of liquid paraffin resorbed from the gastrointestinal tract was possible in a patient with a left chyle fistula in the neck. After a dose of 50 g liquid paraffin administered as a laxative, 246 ml chyle was collected within the following 14 h which yielded a total of 4.5 mg liquid paraffin. Its composition was identical with the administered laxative. Assuming a daily lymph volume of 1.5 l, the resorbed amount would correspond to a resorption rate of 0.5 $^o/_{oo}$ liquid paraffin. The importance of these results as well as the diagnostic consequences arising from the described analytical technique are discussed in detail.

INTRODUCTION

Since the first description by Laughlen [1] in 1925, more than 400 cases of mineral oil (synonyms: liquid paraffin, liquid petrolatum) pneumonia have

been reported in the literature up to 1953 [2] and continue to be described [3]. There is strong evidence, however, that paraffin oil pneumonia occurs much more frequently. Many cases either remain unknown, especially those with only slight pulmonary affection, which are often free of complaints, or are misinterpreted (even in the presence of severe pulmonary involvement [2]) as some other pulmonary disease, such as chronic pneumonia, sarcoidosis, tuberculosis, bronchiectasis, fungus disease or neoplasm (paraffinoma) [4]. Consequently, exogenous lipid pneumonia is commonly an accidental discovery by the pathologist [5]. Intra vitam diagnosis without surgical intervention has only been described exceptionally. Because none of the clinical features is strongly characteristic, the suspicion of mineral oil pneumonia or pulmonary paraffinoma is in most cases only raised by a medical history of paraffin uptake, often unrestricted, and usually in the form of laxatives, oily nose drops or throat sprays. The discovery of lipophages [4,6] in the sputum is not specific, and they are often absent [7]. Histochemical staining methods are only applicable if mineral oil is present in the form of readily visible droplets and none of the various dyes used for lipids is strongly specific for mineral oil [8].

Other methods for the identification of mineral oil have been infrared spectrometry [9—11], nuclear magnetic resonance (NMR) spectrometry [12,13], mass spectrometry [14, 15], column chromatography [16] and thin-layer chromatography (TLC) [7,8]. Though these techniques are virtually specific, they are not valid for reliable quantitative analysis or composition studies of mineral oil preparations ingested for medical purposes and extracted from human or animal tissues under special conditions. Gas—liquid chromatographic (GLC) analysis of liquid paraffin (which is a very complex mixture composed of some hundred saturated hydrocarbons of varying chain length, including straight-chain and branched-chain alkanes, cyclo-alkanes and polycycloalkanes) is the only method to fulfill these criteria tolerably.

In the presented new case of chronic paraffin oil pneumonia, a report is given for the first time about qualitative and even quantitative studies of mineral oil in sputum, plasma and erythrocytes, supplemented by all other methods known so far. The results are completed by our findings in human chyle which was collected after ingestion of a single 50-g dose of liquid paraffin for the treatment of constipation from a man with a surgically induced fistula in the thoracic duct just in front of the angulus venosus sinister.

EXPERIMENTAL

Mineral oil pneumonia was found in a 19-year-old male. The diagnosis was mainly based on a typical 5-year history of gargling with Presido®*, a 99.9% pure mineral oil (total consumption by the patient 5—6 l) and on the histological appearance of an open lung biopsy specimen. Details about the clinical, radiographic and histological findings in this patient will be reported in a separate paper together with new therapeutic approaches [17].

During hospitalization the patient was instructed in breathing exercises and in intermittent exaggerated coughing (although there was no spontaneous

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*Paraffin. perliquid. 1000.0, p-aminobenzoic acid ethyl ester 0.04. Ol. citri 1.0.

cough) in order to obtain as much expectoration as possible. Prior to this regimen the aspiration of mineral oil (Presido) had been stopped. He continued these activities subsequently as an outpatient.

Saturated hydrocarbon standards were purchased from WGA (Düsseldorf, G.F.R.). All chemicals used were of analytical grade. Solvents freshly redistilled before use were free of hydrocarbon contaminants as tested with all the analytical methods mentioned below.

Extraction and isolation of mineral oil

The volume of each sputum sample (10—150 ml/day) was measured. The sputum was sonified twice for half a minute with maximum energy (Branson-Sonifier) followed by lyophilization. The lyophilisate was extracted once with 150 ml hexane—benzene 1:1 (v/v) for 30 min under reflux conditions. The suspension was filtered and the residue discharged. As proven by eight preceding experiments, more than 99% of the mineral oil content of the lyophilized sputum samples were obtained by this extraction procedure so that further extractions were not necessary. The extract was taken to dryness, redissolved in a small volume of hexane and completely transferred into small PTFE-lined screw cap vials. The solvent was evaporated in a stream of nitrogen. A 100-μl volume of an *n*-undecane (500—600 μg) internal standard, dissolved in hexane, was added and the vial was screwed up, ready for quantitative GLC analysis. Some of the crude sputum extracts were dissolved in 20 ml hexane and divided into two parts. One part was used for further quantitative GLC analysis, and the other pooled for additional analytical experiments.

As demonstrated by TLC on silica gel H coated analytical plates (Merck, Darmstadt, G.F.R.) carried out in the solvent system dichlorethane—methanol (98:2, v/v), sprayed with 50% sulfuric acid after development and charred at 150° for 30 min, the sputum extract contained (besides mineral oil) some lipid contaminants. For further detailed identification studies, these contaminating neutral lipid components were separated from the supposed hydrocarbon fraction by column chromatography. Separation was performed on a 20 × 2.5 cm O.D. glass column packed with 10 g Florisil® (magnesium silicate; C. Roth, Karlsruhe, G.F.R.) slurried in hexane. After application of the crude pooled sputum extract dissolved in hexane on top of the column, a thin-layer chromatographically pure hydrocarbon fraction was eluted from the column with a 100 ml portion of hexane. Recovery rates for this analytical procedure, tested with 100—1500 mg samples of Presido, were 98 ± 2%. The hexane eluate was evaporated to dryness. The residue was used for further investigations. A 500 mg sample of Presido, the gargle preparation that our patient had chronically aspirated, was suspended by sonication in distilled water, lyophilized, extracted and purified in the same manner as reported for the pooled sputum sample in order to get sufficient material for comparison purposes.

For examination of saturated hydrocarbon blood levels, a 100 ml sample of anticoagulated blood (ACD) was obtained from our patient and processed immediately. After centrifugation the plasma was decanted and preserved. The cellular residue was thoroughly washed three times with cold isotonic saline. The supernatant was discharged. Plasma and washed red cells, including leucocytes and platelets, were then analyzed following the extraction and isolation procedures as described above.

Since it has been assumed that mineral oil is absorbed from the gastrointestinal tract, we collected all the lymph discharged from a fistula of the ductus lymphaticus which had been introduced in a male patient in the course of a neck dissection operation. Immediately after ingestion of a 50 g dose of liquid paraffin for the treatment of constipation, we collected the patient's chyle over a 14-h period, divided into three successive fractions. The fractions amounted to 46 ml (4 h), 58 ml (4 h) and 142 ml (6 h), respectively. Each chyle fraction was finally subjected to the complete analytical program for mineral oil determination.

Identification and quantitation of mineral oil

A comparative thin-layer chromatogram of the crude unpurified sputum extract and of the purified remedy Presido was developed with hexane on a silica gel H plate. Spots became visible after spraying with 50% sulfuric acid and charring.

Ultraviolet spectra of the purified sputum extract as well as of the purified Presido, recorded with a Beckman Spectralphotometer, Model DB, did not show any ultraviolet absorption, a finding which is typical for saturated hydrocarbons which are free of contaminants.

Infrared spectra of purified sputum extract and of the purified mineral oil "remedy" were recorded between NaCl-plates with a Perkin-Elmer spectrometer, Model IR 225.

[1] H-NMR spectra of the two purified samples were registered with the JNM-MH-100 spectrometer (JEOL) at room temperature in hexadeuterobenzene ($C_6 D_6$) as solvent with tetramethylsilane (TMS) as internal reference.

Mass spectra of the identical samples used for infrared spectrometry and NMR analysis were recorded with the MAT III spectrometer (Varian).

GLC analysis

A Hewlett-Packard gas chromatograph, Model 5830A, equipped with a dual flame ionization detector (FID) and an integrator was used for all analyses. For quick routine analysis the chromatograph was fitted with two 50 cm X 1/8 in. O.D. stainless-steel columns packed with 3% SE-30 on Chromosorb Q, 100—120 mesh. All packings were self-prepared. The columns were filled with packing material under vacuum and gentle vibration. Oven temperature was kept constant for 1 min at 90° and then programmed from 90° to 253° at a rate of 30°/min. The maximum temperature of 253° was maintained for a further 9 min before cooling. Recorded analysis time for the crude mineral oil extract derived from sputum, plasma, blood cells and chyle was 13 min. Injection and detector temperature was 280°. The sample size was 0.1—1.0 μl. An 11-ml nitrogen flow was used as carrier gas. Trying a series of column lengths (1/2 m; 2 m; 3.6 m) filled with different packings of low (Apiezon L; SE-30; OV-101) and intermediate (OV-17; OV-210) polarity, we selected SE-30 coated packings in a 50 cm X 1/8 in. O.D. column for the rapid analysis. This non-polar stationary phase, which is resistant to a temperature of 300°, gave the desired separation of the combined peak for saturated hydrocarbons of varying chain lengths (including straight and branched-chain alkanes, cycloalkanes and polycycloalkanes) from *n*-undecane, and cholesterol peaks when programmed over a

wide temperature range. In order to save time when working with our analytical program for a great number of samples, it was advisable to sacrifice as much column efficiency as possible without reasonable variation of reproducibility.

In order to establish the GLC recovery of liquid paraffin we used six standard mixtures (a–f) composed of n-undecane (0.5 mg) as internal standard, purified Presido (a = 5 mg; b = 10 mg; c = 20 mg; d = 30 mg; e = 40 mg; f = 50 mg) and cholesterol (a = 0.05 mg; b = 0.1 mg; c = 0.2 mg; d = 0.3 mg; e = 0.4 mg; f = 0.5 mg), each standard mixture running ten times. Though we used an internal standard it became necessary to correct our results following GLC analysis, expressed as mean value of a triplicate determination by a factor of 1.165. This factor was the result of an overall decreased recovery of liquid paraffin amounting to 16.5% ± 2.4% S.D. However, the decreased recovery rate was unreal and the results had to be adjusted by a standard factor, because n-undecane did not fulfill the attributes of an ideal internal standard, which besides others should have nearly the same retention time as the sample.

The individual reproducibility of wt.%, expressed as maximum percent deviation of the mean value of ten chromatograms, was 0.2% and 4.6%, respectively. All quantitative results given here for liquid paraffin are mean values of a triplicate determination, corrected by a factor of 1.165.

The following conditions were required for a column to give the best efficiency in composition studies of the complex hydrocarbon mixtures extracted from the different investigated tissues: 2.2 m × 1/4 in. O.D. coiled glass column packed with 12% Apiezon L on Gas-Chrom Q, 100—120 mesh; oven temperature 70—300°, programmed at a rate of 4°/min; injection and detector temperature 300°; carrier gas: nitrogen, flow-rate 28 ml/min (Fig. 5).

RESULTS

According to some earlier reports [7,8] TLC of crude lipid extracts using hexane as solvent system represents a simple and rapid technique for the detection of saturated hydrocarbons. As shown in Fig. 1A this technique has been found effective for the identification of liquid paraffin present in sputum extract and also in all the other human tissues under consideration (plasma, blood cells, chyle). TLC can even be used for the semi-quantitative estimation of saturated hydrocarbons (Fig. 1B), provided that these hydrocarbon mixtures are of approximately adequate composition in comparison to the reference. This limitation is worth mentioning because n-alkanes are almost completely resistant to treatment with sulfuric acid. Other paraffin components can be sufficiently charred, but spot size and intensity are not strongly proportional when equal quantities of differently defined branched-chain or cyclic hydrocarbon standards are compared.

For more detailed identification the crude lipid extracts (sputum, plasma, blood cells, chyle) were purified by column chromatography. The isolated purified non-polar lipid samples were used for several spectrometric analyses. The spectra in Figs. 2—4 are representative of all purified extracts (sputum, plasma, blood cells, chyle) and not only for the pooled purified sputum sample. The infrared spectra in Fig. 2 show identical absorption peaks at 1380 cm⁻¹ (7.27 μ), 1460 cm⁻¹ (6.83 μ), 2860 cm⁻¹ and 2925 cm⁻¹ for the liquid paraf-
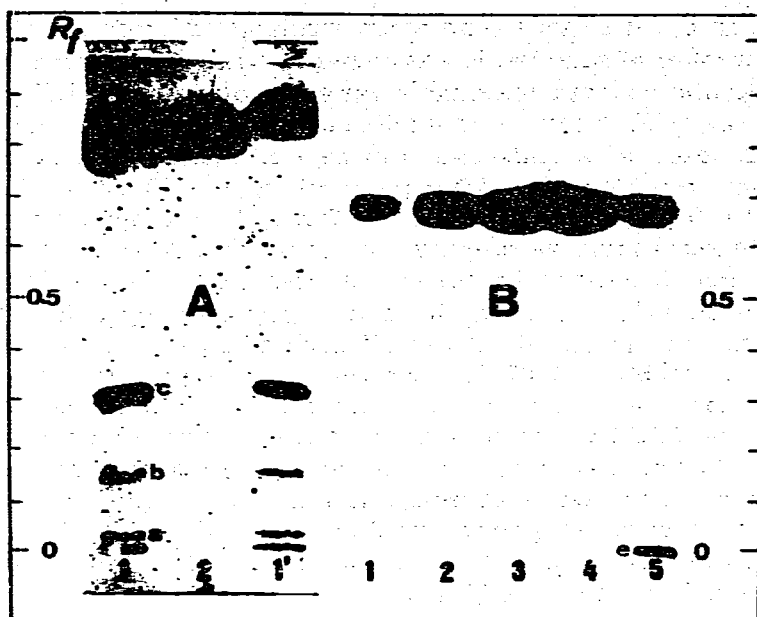
Fig. 1. (A) Thin-layer chromatogram of lipids extracted from sputum in a case of paraffin oil pneumonia (spots in 1 and 1′) compared with a standard of pure paraffin hydrocarbons (spot in 2) identical with Presido: a = monoglycerides; b = free fatty acids; c = cholesterol; d = cholesteryl esters and saturated hydrocarbons
(B) Semi-quantitative thin-layer chromatograms of purified saturated paraffin hydrocarbons (Presido) compared with the crude lipid extract from sputum: 1 = 50 μg; 2 = 100 μg; 3 = 150 μg; 4 = 200 μg (all Presido); 5 = sputum extract (hexane—benzene 1:1), e = lipid contaminants. For analytical conditions see Experimental.
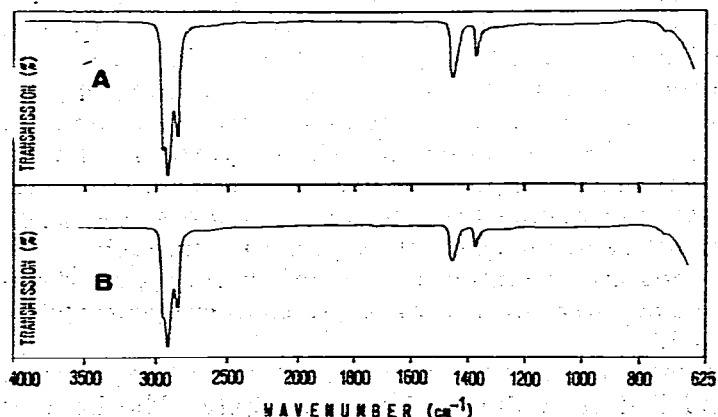

Fig. 2. Infrared spectra of purified Presido (A) and of purified hydrocarbons extracted from sputum (B). For details see Experimental.
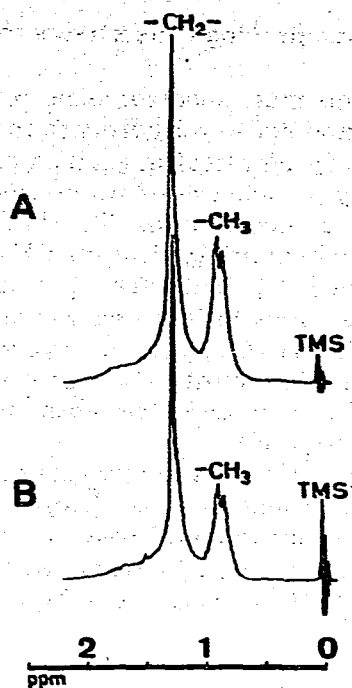
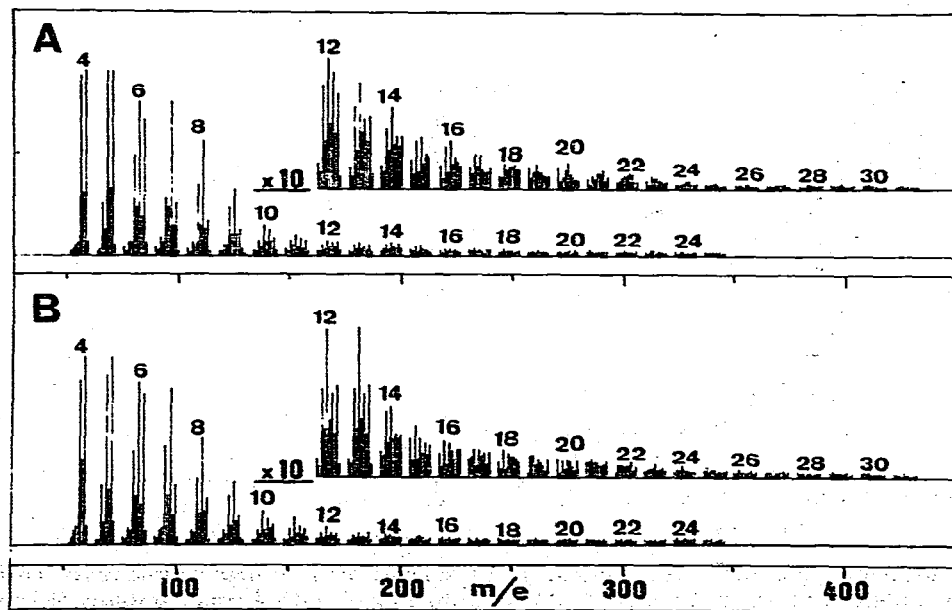Fig. 3. ¹H·NMR spectra of the same samples as recorded in Fig. 2. For details see Experimental.

Fig. 4. Mass spectra recorded from (A) purified Presido and (B) purified sputum extract. For details see Experimental.

fin standard (A) and for sputum hydrocarbons (B), thus proving the extracts to be liquid paraffin.

Figs. 3 and 4 show the [1]H·NMR spectroscopic and mass spectrometric results of the analyses of a liquid paraffin standard (A) and sputum hydrocarbons (B). Comparison reveals that the spectra are completely identical in each case, except for some insignificant differences in intensity which are of no consequence in interpretation because there is a mixture of compounds. The [1]H· NMR spectra show two signals at 1.27 and 0.87 ppm which are typical for CH_2 and terminal CH_3 groupings of a long-chain paraffin [12]. The mass spectra (highest observed mole peak at 436) demonstrate the characteristic fragmentation pattern: a stepwise split-off of CH_2-groups of linear ($C_n H_{2n+2}$) or cyclic ($C_n H_{2n}$) hydrocarbons [12]. In accordance with the iodine number being zero, the NMR spectra as well as the infrared spectra of the investigated extracts rule out the presence of unsaturated hydrocarbon compounds.

Due to abnormal paraffins, the resolution of the purified tissue extracts on GLC (Fig. 5) was poor, so that only a few n-alkanes, identified by means of pure standards, became recognizable from the typical unresolved broad peak. Chromatograms recorded from pooled sputum extract as well as from many individual sputum samples showed quite the same pattern as did the liquid
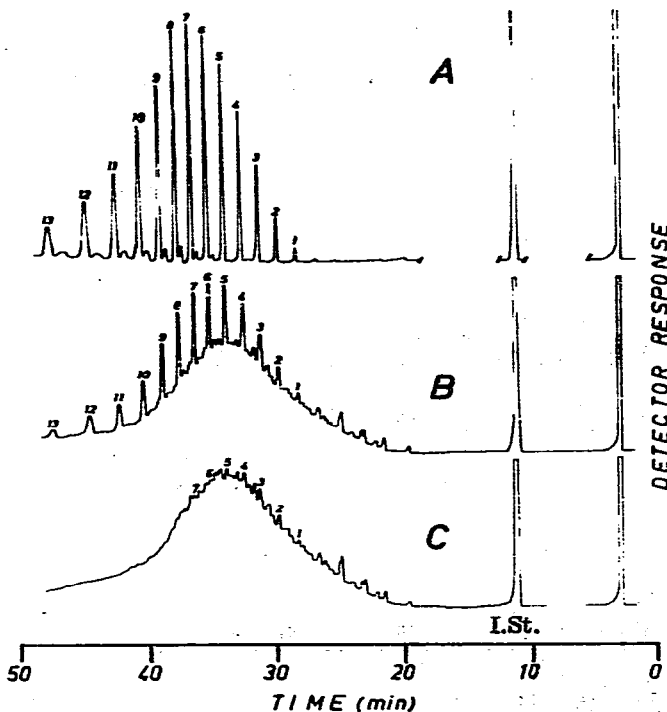

Fig. 5. Gas chromatograms for detailed composition studies of: mixtures of n-alkanes used as tissue embedding medium in histology (A); Presido mixed with a small amount of the mixture of n-alkanes (B) and purified sputum extract (C) obtained from the patient with paraffin oil pneumonia. I.St. = internal standard. Numbers above the peaks in A, B and C indicate their identification in the normal alkane series (1 = n-eicosane and 13 = dotriacontane, respectively). For analytical details see Experimental.
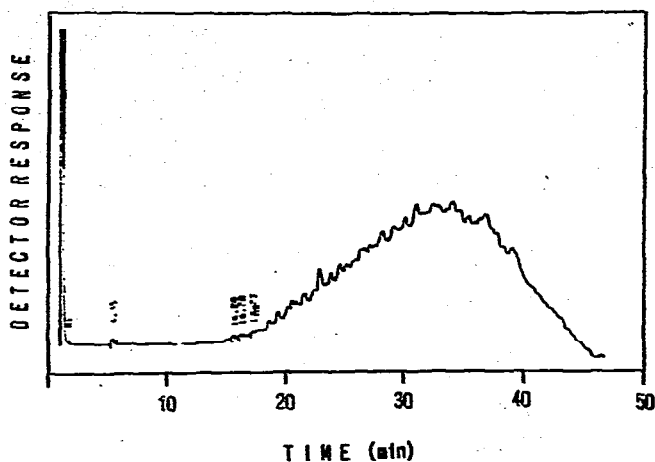
Fig. 6. Gas chromatograms of the purified, saturated hydrocarbon fraction extracted from plasma in the young man suffering from mineral oil pneumonia. For analytical details see Experimental.

paraffin Presido. A GLC pattern quite similar to the sputum extracts could be recorded from the non-polar lipids derived from the patient's plasma (Fig. 6) and blood cells and from the chyle extract (Fig. 7).

Quantitative GLC analyses (Fig. 8) yielded a daily expectoration rate of liquid paraffin within the range of 4.1 mg—79.5 mg measured over a period of 233 days. Paraffin plasma levels were 1.3 mg%. The corresponding value for the thoroughly washed cellular blood residue was 1.6 mg%. The total mineral oil content of the analyzed 246 ml chyle volume, collected within a 14-h period
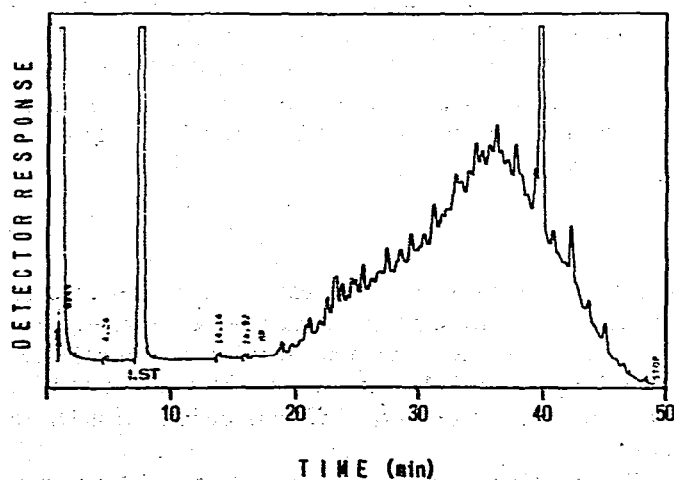

Fig. 7. Fractogram of the purified, saturated hydrocarbon mixture derived from the chyle of a man with a fistula of the thoracic duct. Chyle was collected within 14 h of the ingestion of a 50 g dose of liquid paraffin. I.St = internal standard. For analytical details see Experimental.
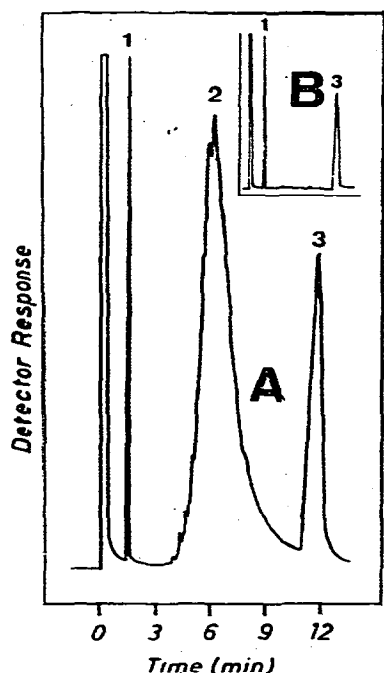
Fig. 8. Quantitative rapid GLC record of a complex mixture of saturated hydrocarbons (2) containing sample extracted from sputum (A) with n-undecane as internal standard (1) and cholesterol (3). A sputum sample of a healthy control person is shown in (B). For analytical details see Experimental.

after the ingestion of mineral oil, amounted to 4.5 mg. This value is composed of 0.9 mg, 2.2 mg and 1.4 mg moieties, respectively, when enumerated in the actual collection order of lymph fractions. Since we are quite sure that only a small volume of the chyle formed during the 14-h period could be collected from the observed patient for analytical purposes, we assume that the real quantity of mineral oil absorbed by the human intestinal tract after ingestion is much greater than was measured here.

DISCUSSION

Our results show that GLC is a very suitable method for the qualitative and quantitative demonstration of saturated hydrocarbon mixtures in various human tissues and secretions. It is the only one of the numerous analytical methods for the demonstration of hydrocarbons discussed in this paper to permit, to a limited degree, a characterization and differentiation of simple and complex hydrocarbon mixtures.

The saturated hydrocarbons of vegetable and animal origin consist mainly of n-alkanes, with the exception of some fish liver oils which contain considerable amounts of branched-chain alkanes [8]. In contrast, the saturated hydrocarbons found in mineral oil (a defined refinery product obtained from petroleum) contain considerable amounts of very heterogenous cycloalkanes and

polycycloalkanes. It is this very complex portion that distinguishes mineral oil from other saturated hydrocarbon mixtures. Its presence in biological material, which can be unequivocally proven only by GLC, permits the definite conclusion that liquid paraffin has been incorporated. This incorporation can be due to the administration of paraffin oil preparations for medical purposes but also to the ingestion of food since these preparations are frequently used as additives in the food processing industry [18]. The chemical composition of the liquid saturated paraffins which are ingested by humans in rather considerable quantities (an annual per capita consumption of 47.5 g has been estimated for the U.S.A. in 1964 [18]) is by no means completely identical but subject to wide fluctuations, depending on the composition of the crude petroleum which varies from oil field to oil field [19].

This is demonstrated by Figs. 6 and 7 in which small, but distinct and reproducible differences can be recognized in the composition of the liquid paraffin used as a laxative and that of the gargling preparation, Presido. Furthermore, GLC has the advantage that the crude lipid extracts obtained do not have to be purified for the demonstration of hydrocarbons, whereas this is essential for all the other methods described.

The demonstration of complex paraffin in the sputum permits the rapid and definite diagnosis of paraffin oil pneumonia or pulmonary paraffinoma without resorting to lung biopsy. The possible demonstration of lipophages in the sputum [4,6] is no definite proof of the existence of mineral oil pneumonia or pulmonary paraffinoma because the lipophages may be missing. This was the case with our patient, although his expectoration of mineral oil was up to 79.5 mg daily. Likewise, the detection of pulmonary mineral oil deposits in lung biopsy material by the pathologist can at most lead to the tentative diagnosis of exogenous lipid pneumonia since there is no strongly specific dye to permit the definite differentiation of mineral oil from other lipids [8].

Since GLC also yields reproducible quantitative results, it can be helpful in testing various forms of treatment in cases of mineral oil pneumonia. As far as equipment is concerned, an integrator system permitting peak summation is necessary for a quantitative analysis. The demonstration of typically composed complex hydrocarbons in the plasma and blood cells of our patient with mineral oil pneumonia, more than a year after the long-term use of mineral oil, seems to indicate that small quantities of paraffin are being removed from the involved lung segments, probably via the lymphatic channels of the lung. It is also conceivable that the liquid paraffin, which is always deposited in the mesenteric lymph nodes, the spleen and the liver after prolonged oral ingestion of paraffin oil [9] finds its way from these organs into the bloodstream, or that the resorbed liquid paraffin is directly carried into all organs with the bloodstream. The latter assumption is supported both by the postulation of previous authors [20—24] that ingested paraffin oil is resorbed to a considerable extent from the gastrointestinal tract via the lymphatic channels, and by the results of our own first exact measurements in man. Assuming that the daily human lymph volume amounts to an average of 1.5 l (range: 0.5 l—3.0 l) [25], the linear conversion to a total volume of 1.5 l would result in a resorption rate of approximately 0.5°/oo of liquid paraffin. This does not take into account that considerably more liquid paraffin can probably be resorbed with

a high-fat diet because emulsifying conditions are better [21]. Although the estimated resorption rate of 0.5% in humans is still far below that of 2% measured by Ebert and coworkers [26] with tritiated mineral oil in rates, it is of some importance because there is no evidence that complex saturated hydrocarbons can be metabolized by the human or animal organism. Contrary to medium- or short-chain-n-alkanes, which can be oxidized to fatty acids by the animal organism [23,27,28], they are deposited unchanged into the human reticuloendothelial system and sometimes suggest follicular lipidosis of the spleen to the attentive pathologist [10]. That such exogenous lipidosis is not always without clinical importance is demonstrated by the case study of Nochomovitz et al. [9] of a 54-year-old man who died from severe malnutrition which probably resulted from the unrestricted use of paraffin oil as a laxative for many years.

The use of liquid paraffin oil in medicine should be restricted to a few clearly indicated fields, e.g. treatment of intoxications resulting from certain organic solvents.

REFERENCES

1 G.F. Laughlen, Amer. J. Path., 1 (1925) 407.
2 R.W. Jampolis, J.R. McDonald and O.T. Clagett, Int. Abstr. Surg., 97 (1953) 105.
3 R.E. Scully, J.J. Galdabini and B.U. McNeely, New Engl. J. Med., 296 (1977) 1105.
4 J.M. Hoffard, Med. J., 40 (1968) 340.
5 C.W. Elston, Arch. Dis. Childhood, 41 (1966) 428.
6 S. Losner, B.W. Volk, W.R. Slade, L. Nathanson and M. Jacobi, Amer. J. Clin. Path., 20 (1950) 539.
7 P.M. Bayer, R. Dudczek and H. Fuchs, Wien. Klin. Wochenschr., 86 (1974) 438.
8 J.K. Boitnott and S. Margolis, Bull. Johns Hopkins Hosp., 118 (1966) 402.
9 L.E. Nochomovitz, C.J. Uys and S. Epstein, S.Afr. Med. J., 49 (1975) 2187.
10 H.G. Rose and A.F. Liber, J. Lab. Clin. Med., 68 (1966) 475.
11 W.D. Wagner, P.G. Wright and H.F. Stikinger, Amer. Ind. Hyg. Ass. J., 25 (1964) 158.
12 D.H. Williams and J. Fleming, Spektroskopische Methoden in der organischen Chemie, Georg Thieme Verlag, Berlin, 1971.
13 R.B. Williams, in E. Thornton and H.W. Thompson (Editors), Proc. Institute of Petroleum Hydrocarbon Research Group, Conference on Molecular Spectroscopy, Pergamon Press, New York, 1959, p. 26.
14 J.K. Boitnott and S. Margulis, Bull. Johns Hopkins Hosp., 118 (1966) 414.
15 R.F. Riley, Y. Hokaus and P. Kratz, Cancer Res., 18 (1958) 825.
16 J.J. Wren, Chromatogr., Rev., 3 (1961) 111.
17 H. Heckers, F.W. Melcher, K. Dittmar, K. Knorpp and K. Nekarda, Lung, in press.
18 Nutrition review, 25 (1967) 46.
19 A.F. Holleman and F. Richter, Lehrbuch der organischen Chemie, Walter de Gruyter & Co., Berlin, 1961, p. 28.
20 K. Bernhard and E. Scheitlin, Helv. Physiol. Acta, 10 (1952) 54.
21 A.C. Frazer, H.J. Schulman and H.C. Stewart, J. Physiol. (London) 103 (1944) 306.
22 A.C. Frazer, H.C. Stewart and J.H. Schulman, Nature (London), 149 (1942) 167.
23 D. Stetten, J. Biol. Chem., 147 (1943) 327.
24 W.A. Stryker, Arch. Path., 31 (1941) 670.
25 A.E. Dumont and J.H. Mulholland, New Engl. J. Med., 263 (1960) 471.
26 A.G. Ebert, C.R. Schleifer and S.M. Hess, J. Pharm. Sci., 55 (1966) 923.
27 K. Bernhard, U. Gloor and E. Scheitlin, Helv. Chim. Acta, 35 (1952) 1908.
28 R.D. McCarthy, Biochim. Biophys. Acta, 84 (1964) 74.