MEDICAL RESEARCH COUNCIL
INSTITUTE OF PETROLEUM 1955 -

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P.O. BOX 45, LINDEN, N.J.

MEDICAL RESEARCH DIVISION

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June 21, 1957

Mr. D. V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

I am attaching some information forwarded to me from Dr. M. N. Nesquist by Mr. A. E. Dooley. Perhaps you would like to have this duplicated and distributed to the members of the Subcommittee on Carcinogenicity and also to Dr. Horton and Dr. Kehoe.

Sincerely yours,

R. E. Eckardt cjh

R. E. ECKARDT, M. D.

REE:cjh
Attach. - Institute of Petroleum. Research-Group No. 2. Minutes of Meeting No. 17 held at 26, Portland Place, W.1. on the 12.3.57.

Copy From D. V. Stroop For Information Of Members Of Subcommittee on Carcinogenicity 6/26/57

INSTITUTE OF PETROLEUM RES-2-17

RESEARCH GROUP NO. 2.

Minutes of Meeting No. 17 held at 26, Portland Place, W.1. on the 12.3.57.

ATTENDANCE

IN THE CHAIR:	F. Morton
MEMBERS:	S.J.M. Auld G.A. Dickins J.N. Haresnape C.F. McCue H.W. Stevenson
APOLOGIES FOR ABSENCE:	B.G. Banks J.W. Barrett J.W. Cook A. McLean
SECRETARY:	E. Herbert

1. MINUTES OF MEETING NO. 16. 23.10.56:

These were signed as correct, subject to the addition of "pages 146-155" to "The Transactions of the Institution of Chemical Engineers" Vol. 34 No. 2. (See Minute No. 7) and the alterations of "Research No. 2 Committee" to "Research Group No. 2."

2. MATTERS ARISING:

(a) The Chairman mentioned that the paper for publication in the IP Journal in continuation of that presented at the World Petroleum Congress had been prepared (See Minute 3 of Meeting 15).

(b) The disappearance of activity referred to by Dr. Irwin was being studied in the present series of tests at Birmingham (See Minute No. 5 and Minutes No. 4 para. 2 of M.R.C. Minutes M.R.C.57/176 and C.A.M.O. Minute 21).

3. CONSIDERATION OF FINANCE FOR FUTURE PROGRAMME:

The Chairman's memorandum and the report on the Constitution and Biological Activity of Mineral Oils which had been circulated was noted.

The Chairman mentioned that there was no great urgency for additional financial support as the I.C.I. and the Salter Research Institute had made contributions of £500 each and the M.R.C. Current Budget included a grant similar to that made last year. It appeared that there was sufficient finance available to complete the present work. During general discussion, it was evident that development of methods was of more interest to industry than actual results and that a biological testing unit for use of industry would be welcome. The Chairman undertook to prepare a memorandum of suggestions for future work which would include (a) programme for Middle Distillates and (b) Oxidation.

Members were asked to write their comments to the Chairman after consideration of his memorandum so that recommendations which were likely to receive industry's financial support could be forwarded, through the Research Committee, to IP Council early in 1958.

4. DATE OF NEXT MEETING:

To be arranged by the Chairman.

May, 1957. JB.

MEDICAL RESEARCH COUNCIL

For Specified Circulation
(Committee and Council only)

MRC.53/70
CAMO.53/1

Committee on the Carcinogenic Action of Mineral Oils

REPORT ON THE

FIRST THREE YEARS' WORK

MEMBERS OF THE COMMITTEE

Professor T. Ferguson, D.Sc., M.D., F.R.C.P.E., D.P.H. (Chairman)

Colonel S. J. M. Auld, O.B.E., M.C., D.Sc.

Professor J. W. Cook, D.Sc., F.R.S.

Professor A. Haddow, M.D., D.Sc.

I. Hieger, D.Sc.

J. O. Irwin, D.Sc.

Professor F. Morton, Ph.D., F.R.I.C.

* Professor R. D. Passey, M.C., M.B., D.P.H.

Professor J. R. Squire, M.D., F.R.C.P.

D. L. Woodhouse, Ph.D., F.R.I.C.

B. S. Lush, M.D., M.R.C.P. (Secretary)

* Professor Passey was not a member of the Committee during the period referred to in this Report.

Section I

INDUSTRY AND RAW MATERIALS

The petroleum industry and skin cancer

The petroleum industry has been concerned with occupational skin cancer caused in the following ways:

(1) From long-term contact of lubricating oils with the skin of workers (e.g. mule-spinners' cancer).

(2) From long-term contact of constituents of unrefined paraffin wax with the skin of workers (shale workers' cancer).

(3) From long-term contact of cutting oils with the skin of workers (e.g. skin cancer in the light engineering industry).

(4) From other possible miscellaneous causes (e.g. skin cancer in furnace workers).

The first three of these groups have been confirmed in the laboratory by animal experiment. In addition animal experiment has shown that selective solvent extracts and cracked oils of low volatility may cause skin cancer, but this has not been noted in man in industry.

The light oils and wax of shale have long been known as causes of skin cancer. It is most likely that this effect is due to carcinogens accompanying the waxy fractions, and not to the wax itself, which is paraffinic in nature. Recognition of the factors concerned has led to greatly improved conditions of work in the shale oil refineries and has brought the disease largely under control. There is no recorded corresponding occurrence of skin cancer or of dermatitis amongst mineral oil refinery workers.

The occupational disease of mule-spinners' cancer has attracted much attention in this country over the last thirty or so years and from early on was ascribed to the lubricating oil used. Investigation by a committee set up by the Manchester Corporation in 1928 confirmed this view and resulted in an attempt on their behalf by C. C. Twort six years later to distinguish, by physical means, carcinogenic from non-carcinogenic oils, using a formulation based upon a relationship involving specific gravity and specific refractivity. The formula was wrongly accepted in many quarters as a solution of the problem and lead to a false sense of security until Auld in 1938[1] pointed out its weaknesses. Between 1934 and 1939 an independent examination of C. C. and J. M. Twort's data was carried out by Irwin[2], who while finding considerable correlation between carcinogenic potency and refractivity, did not confirm all the conclusions in C. C. and J. M. Twort's published papers. The Manchester formula had also led, unfortunately, in the minds of many petroleum scientists, to insufficient attention being paid in the textile industry to cleanliness, working conditions,

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1. Auld, S. J. M., (1938) Specific refractivity and carcinogenicity of mineral lubricating oils. J.Inst.Pet.Tech. 24.577.

2. Irwin, J. O. & Goodman, N. (1946) J.Hyg., Camb. 44, 362.

medical inspection, change of occupation with long-term medical follow-up for susceptible workers and so on; that is to say, the measures which had brought the paraffin dermatitis and paraffin cancer of the shale industry under control. It also led to insufficient attention being paid to the development of truly non-carcinogenic oils. It had been apparent in the petroleum industry that more drastic refining, such as solvent extraction or sulphuric acid treatment, which might be expected to destroy or eliminate possible carcinogens, was the most logical approach to the problem as far as lubricating oils were concerned. In addition, acceptance of the formula delayed consideration of systematic examination of petroleum for the purpose of separating possible carcinogens and study of their structure and properties.

In 1943-44 attention was focussed on the lubricating oil extracts which were being used as substitutes for extenders, plasticisers, etc., which could no longer be obtained under war-time conditions. It was suggested that these extracts might have carcinogenic or other very noxious properties and, as a result, work was initiated at Birmingham University by the Petroleum Board for the biological testing of extracts. This work is referred to later, and more fully, in Section III of this report[3]. If the supposition that by solvent refining oil stocks from which lubricating oils are made, the extracts are made more carcinogenic than the original stocks, then the refined oils must be less carcinogenic, which leads to the thought that intensive refining to the stage where white oils are produced would be a logical means of obtaining a non-carcinogenic oil. In 1946 the Petroleum Board took the step of recommending that if a prima facie case could be made out for the non-carcinogenicity of such white oils, they should be made available to the textile trade as mule spindle oils. At a meeting convened by the Ministry of Labour, the decision of the Ministry of Fuel and Power to make white oils available as textile lubricants was confirmed, despite the meagre evidence available and despite their scarcity and dollar cost at that time. Simultaneously, the Petroleum Board announced its further financial subsidy for research work at Birmingham to supplement the very limited information on which the decision had been made.

In 1948, the master spinners and the cotton operatives recalled the ad hoc committee of 1946 to consider the matter, by which time there was corroboration from Birmingham of the biological inactivity of white oil samples submitted by the Petroleum Board. Decision was thereupon made to standardize on white oils, subject to practical tests showing them to have satisfactory lubricating properties. Mill trials carried out with plain white oils and white oils blended with fatty oils, all of comparable viscosity, showed satisfactory performance and very little difference between the oils in power consumption. The ad hoc committee which achieved an official basis by being brought under the control of the Factory Department of the Ministry of Labour and National Service, then asked the Petroleum Industry representatives to submit a specification to cover suitable white oils for adoption by the Trade. This was done and the specification is referred to in the Ministry of Labour and National Service publication: "Mule Spinners' Cancer" - Second Interim Report of the Joint Advisory Committee of the Cotton Industry (H.M. Stationery Office). One of the recommendations in this report is that appropriate action should be taken to make the specification as effective as possible at the earliest possible date and it is understood that consideration is being given to its enforcement by means of a Statutory Instrument.

The work at Birmingham on behalf of the Petroleum Board to evaluate the carcinogenicity of solvent extracts is dealt with in Section III[3] of this report. The results varied quantitatively from one material to another, but showed that solvent refining tended to concentrate carcinogens, where they existed, in the extract. The most active were lower in biological activity than the corresponding coal tar products and less noxious than certain materials already in use for similar purposes. Further work involving biological testing was envisaged and the Board set aside money for this purpose. When the Board was dissolved in 1948, it approached the Institute of Petroleum, with the concurrence of the Ministry of Fuel and Power, to

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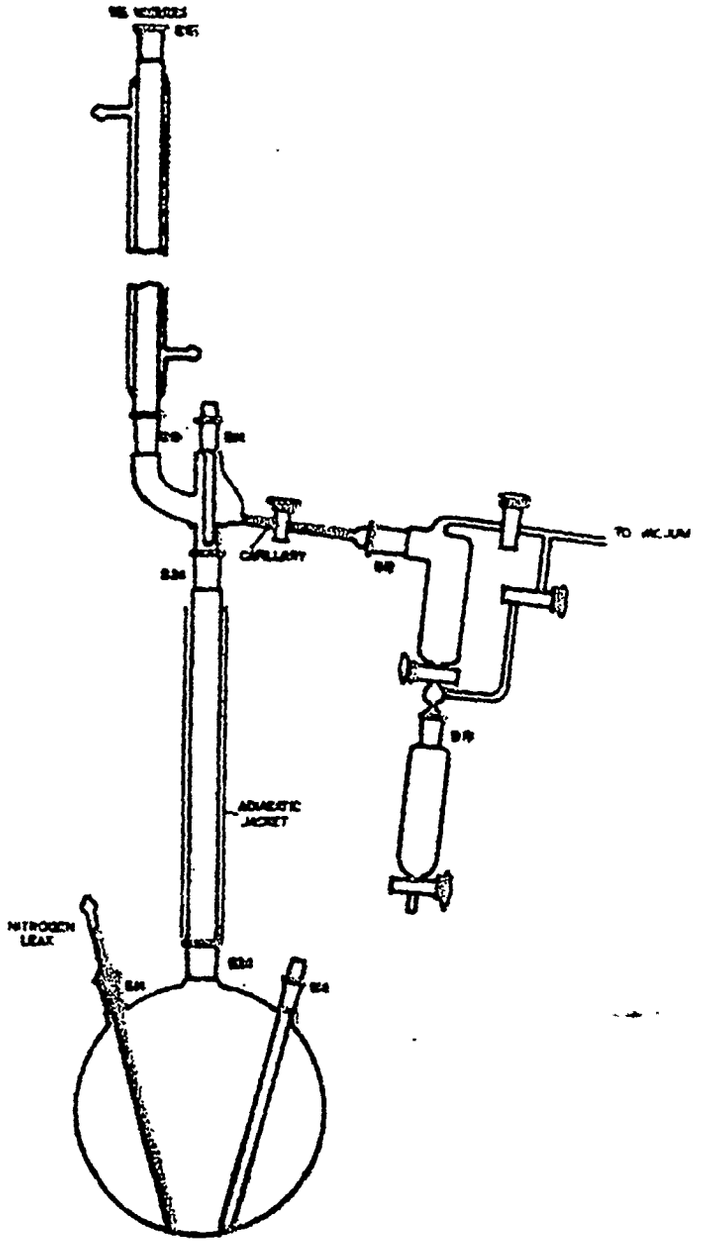
3. See below page 1[illegible].

Fig. 1.



The distillation was repeated with further charges of 1000 ml. until 9000 ml. of each fraction had been distilled. Data for the first distillation of each of the three fractions are given in Tables I, II, and III.

Blending. After discussion with Professor Squire it was agreed to blend the distillates according to the following scheme shown in Table I.

The arrangement gives five fractions from each crude charge, with a minimum column of 900 ml. for any fraction. The final blends and volumes are given in the attached TablesI, II, III, IV.

Report on samples of Lagunillas, Oklahoma and Kuwait distillates received. At the Tenth meeting of the Research Group No. 2 it was agreed that future work on the examination of crude oils should be restricted to the original three crude oils from Lagunillas, Oklahoma and Kuwait. Accordingly, samples of commercially prepared distillates approximating to the No. 4 fraction prepared at Thornton Research Centre have been obtained and are stored at the University of Birmingham.

Lagunillas distillate. Through the kindness of Mr. H. W. Stevenson and the courtesy of the N.V. Curacaosche Petroleum Industrie Maatschappij we have been fortunate to obtain 10 drums of Lagunillas distillate 300-400°C. prepared by commercial vacuum distillation from a charge stock consisting of almost 100% Lagunillas crude oil. The inspection data on this distillate is given in Table V; for comparison, the distillation data on fraction No. 4 prepared at Thornton Research Centre is included.

Oklahoma Bright Crude Distillate. Through the kindness of Colonel Auld and the courtesy of Dr. L. C. Beard Jr., and the Socony-Vacuum Oil Co. 10 drums of No. 70 lubricating oil distillate ex Oklahoma Bright Crude have been received and are stored at the University of Birmingham. Inspection data on this material is given in Table VI with the corresponding distillation data from Thornton Report. It will be seen that in this case the new material is a somewhat higher boiling fraction than the original O_4, the bulk of the distillate being concentrated in the 350-400°C. range. There is, however, sufficient of the material available to prepare large samples in the 300-350°C. range should this prove to be necessary.

Kuwait Vacuum Distillate. It proved impossible to obtain, as a single stream, a sample of Kuwait Vacuum Distillate boiling in the range 300-400°C. Through the kindness of Mr. E. J. Bourman and the courtesy of Esso Petroleum Co., Fawley, we have obtained three drums of each of a vacuum overhead and No. 1 side stream fraction which together cover the boiling range in which we are interested. The distillation data for the two fractions are shown in Fig. 2 with a similar distillation for the Lagunillas distillate for comparison.

Future programme

The erection of the Stedman Vacuum distillation unit is now completed. Thermocouples and manometers etc. are now being calibrated and the adiabatic jacket winding has been fitted in place and is under test. On completion of these tests the unit will be charged with the first of the Kuwait samples and the distillate collected in appropriate size samples. In the first case it is proposed to collect the samples in accordance with the boiling range distribution $K_{4.1}$, $K_{4.2}$, $K_{4.3}$, $K_{4.4}$ and $K_{4.5}$ (cf. Report No. B1/52 - page 3). Material boiling outside this range will be collected separately and stored.

A full report on the design etc. of the Stedman unit will be

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7. This is the text of a report (B.2/52) by Professor Morton to Research Group No. 2 of the Institute of Petroleum and is reproduced by their permission.

accept responsibility for continuing the work, and offered a substantial sum to continue the research. The Institute of Petroleum accepted the responsibility and implemented it by forming (under its Research Committee) the Research Group No. 2., whose Chairman is Colonel Auld. The Research Group No. 2 felt that a fundamental investigation was required in an endeavour to separate the active components present in petroleum and to determine their properties. As there are many different oil-fields and many different crude oils, it was necessary to be realistic and make a limited choice. At about the same time the Medical Research Council's Committee on the Carcinogenic Action of Mineral Oils was set up and it was natural that the activities of the Institute of Petroleum Research Group No. 2 should be dovetailed with those of the Medical Research Council's Committee.

Raw materials

As a result of the co-operation between the Medical Research Council Committee and Research Group No. 2 of the Institute of Petroleum, three crude oils, Kuwait, Lagunillas and Oklahoma were selected as being either typical of crude oils likely to be much in use in the United Kingdom, or alternatively typical sources of lubricating oil fractions, bright stocks and residual lubricating oils. Substantial supplies of these oils were arranged by the oil companies concerned, i.e. Kuwait Oil Co. (Kuwait), Shell Petroleum Co. (Lagunillas) and Socony-Vacuum Oil Co. (Oklahoma). Each oil was initially fractionated by controlled distillation at the Shell Research Centre at Thornton-le-Moors into four fractions and a residue. This fractionation is described in Appendix II at the end of Section IV. The fractions were numbered for convenience O_1, O_2, O_3, O_4, O_5 (Oklahoma), and similarly K_{1-5} and L_{1-5} for Kuwait and Lagunillas respectively. Later, the fourth fraction of each oil was selected for further study[4]. A description of the progressive fractionation of the three chosen fractions, carried out by Professor F. Morton at Birmingham University, follows.

Redistillation of Fractions O_4, L_4 and K_4[5]. Three crude oils, representative of typical stocks, from Oklahoma, Lagunillas and Kuwait respectively, had been fractionated at the Thornton Research Centre into four overhead fractions and a residue. The distillation had been conducted with open steam to avoid the use of temperatures in excess of 250°C. In each case fraction 4 (boiling range approximately 300-400°C. at 750 mm. Hg.) was selected for further investigation, because these fractions had been shown to be the most carcinogenic[6].

The further fractional distillation of each of the three samples O_4, L_4 and K_4 was carried out in the following manner.

Apparatus. The fractionating column and auxiliary apparatus is shown in the accompanying diagram (Fig. 1). The column (18 in. high x 1 in. diameter) was packed with $\frac{1}{8}$th Fenske helices. Adiabatic conditions were maintained by a heating jacket and the flask was heated by isomantle to avoid local overheating.

The still was charged with 1000 ml. of the sample (previously dried over anhydrous sodium sulphate) and operated under total reflux at a controlled pressure of 1.0 mm.Hg. for 1 hr. The take off cock was then opened to give an effective reflux ratio of 10-1. This distillate was collected to 100 ml. fractions, temperature and pressure being recorded throughout. The distillation was stopped at a still pot temperature of 250°C.

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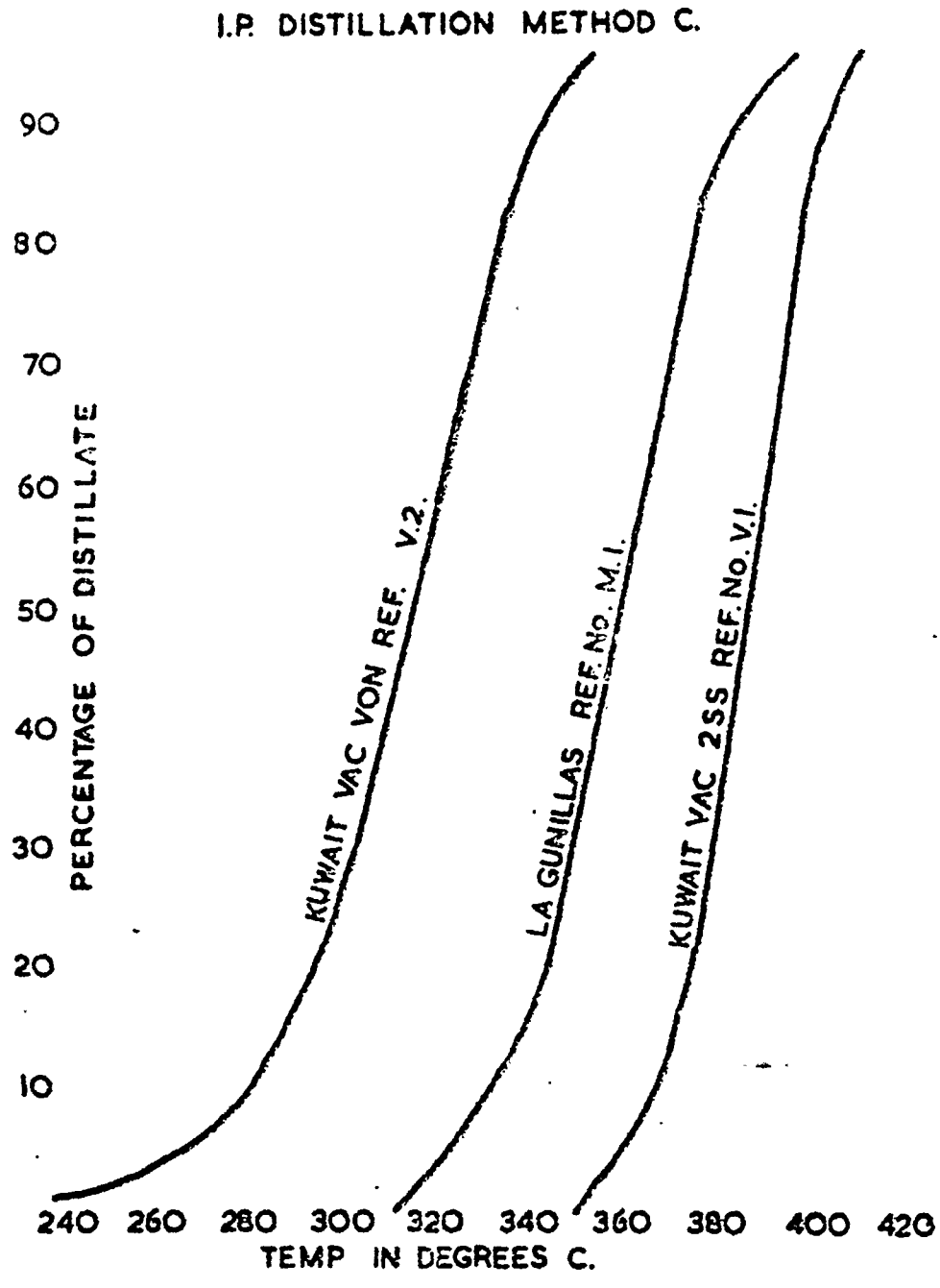
4. Further description of this work is given in Sections II (page 7) and III (page 15) of this report.

5. This is the text of a report (B1/52) by Professor Morton to Research Group No. 2 of the Institute of Petroleum and is reproduced by their permission.

6. See below page 14.

Fig. 2.



N.B.

1. These curves are approximate.
2. The nomenclature is that used by the petroleum companies and does not correspond exactly with that used by the Committee.

Table II. Oklahoma crude fraction No. 4. Volume charge 1000 ml. Pressure 1 mm.Hg.

Fraction No.	Boiling Range °C(Corrected)	Volume ml.
1	[illegible]-290	100
2	290-320	100
3	320-360	100
4	360-370	100
5	370-370	100
6	370-390	100
7	390-400	100
8	400-400	100
9	400-410	100
Residue	410	100

Table III. Laguinillas crude fraction No. 4. Volume charge 1000 ml. Pressure 1 mm.Hg.

Fraction No.	Boiling Range °C(Corrected)	Volume ml.
1	~~6~~-380	100
2	380-385	100
3	385-390	100
4	390-400	100
5	400-400	100
6	400-410	100
7	410-410	100
Residue	410	300

Table IV. Kuwait crude fraction No. 4. Volume charge 1000 ml. Pressure 1 mm.Hg.

Fraction No.	Boiling Range °C(Corrected)	Volume ml.
1	~~8~~-300	100
2	300-350	100
3	350-360	100
4	360-380	100
5	380-385	100
6	385-390	100
7	390-395	100
8	395-400	100
Residue		200

TABLE I

Distillation range and volume of samples prepared for biological testing

Oklahoma (O_4)			Laguinillas (L_4)			Kuwait (K_4)		
Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.	Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.	Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.
$O_{4.1}$	-320	1800	$L_{4.1}$	-380	900	$K_{4.1}$	-350	1770
$O_{4.2}$	320-370	1800	$L_{4.2}$	380-390	1800	$K_{4.2}$	350-380	1690
$O_{4.3}$	370-390	1800	$L_{4.3}$	390-400	1800	$K_{4.3}$	380-390	1760
$O_{4.4}$	390-410	2700	$L_{4.4}$	400-410	1800	$K_{4.4}$	390-400	1070
$O_{4.5}$	Residue > 410	900	$L_{4.5}$	Residue >410	2700	$K_{4.5}$	Residue > 400	2500

TABLE V

N.V. Curacaosche Petroleum Industrie Maatschappij
(Curacao N1A.)

Analysis of a sample of Laguinillas distillate(4)

Specific gravity at 15/4°C	0.9299	
Specific gravity at 60/60°F	0.9304	
Gravity A.P.I. at 60°F	20.6	deg.
Viscosity Redwood I at 140°F	55	sec.
Flashpoint P.M. cc.	330	°F.
Water	0.05	%
Pourpoint	-33	°F.
Organic acidity	5.0	mg.KOH/g.
Inorganic acidity	nil	mg.KOH/g.
Ash	nil	%
Asphaltenes	nil	%
Distillation (Vacuum 1 mm.)	°C	Thornton Report No. C3.2/50 Table 3 fraction 4 °C
2% recovered at	310	31 317
10% " "	332	343
20% " "	342	350
30% " "	350	354
40% " "	357	360
50% " "	365	365
60% " "	371	370
70% " "	376	376
80% " "	383	384
90% " "	394	-
95% " "	401	-

(4) This Table is reproduced by permission of the Institute of Petroleum

N.B.

These samples were prepared by the petroleum companies and are those described on page 4 under the heading "Report on samples of Laguinillas, Oklahoma, and Kuwait distillates".

TABLE VI

Socony-Vacuum Laboratories

Analysis of a sample of 70 distillate(5)

Laboratory No.			934796	
Buffalo Refinery No.			192	
Product			70 distillate	
Gravity			28.2	°A.P.I.
ASTM colour			1-1/2	
Pourpoint			+30	°F
Flashpoint			335	°F
S.U. Viscosity			83	@ 100° F
Vacuum distillation	°F	°C	°F	Thornton Research Centre Report No. C3/2/50 Table II Fraction 4 °C
(Readings adjusted to 760 mm.Hg.)				
	IBP	296	566	296
	10%	356	673	315
	20%	368	695	320
	30%	374	706	327
	40%	378	712	332
	50%	385	725	338
	60%	388	731	344
	70%	392	737	350
	80%	396	745	359
	90%	402	755	375
	95%	405	762	400

(5) This Table is reproduced by permission of the Institute of Petroleum.

N.B.

These samples were prepared by the petroleum companies and are those described on page 4 under the heading "Report on samples of Lagunillas, Oklahoma, and Kuwait distillates".

issued in due course. The unit consists of a still pot capable of a working charge of 75 gals., a fractionating column 10 ft. high, 6 ins. diameter packed with circular Stedman packing, and the necessary condensers, heaters, reflux accumulation and control instruments. The unit is the gift of Messrs. Trinidad Leaseholds Ltd. and the new packing has been supplied by courtesy of Foster Wheeler Corp. and the Midland Tar Distillers Co. Ltd.,

Section II

THE RESULTS OF CHEMICAL STUDIES

Objective

The ultimate objective of the chemical part of the investigation is the identification of the constituent(s) responsible for the carcinogenic action of the petroleum oils and the utilisation of this knowledge in devising chemical or physical methods for the detection and estimation of the carcinogenic constituents of commercial oil fractions. This would furnish a rapid means of evaluating the carcinogenic potency of a given material, thus obviating the need for lengthy and costly biological examinations. Furthermore, a knowledge of the chemical nature of the active material might well lead to the elaboration of refining procedures which would result in detoxification of the oil.

The oil fractions

Three representative crude oils were selected (see Section I). Each was separated by distillation at the Thornton Research Centre into four fractions and a residue. As it is known that very high temperatures (ca 600-1000° C) may transform such materials into carcinogenic polycyclic hydrocarbons, the conditions of distillation were carefully controlled so as to eliminate any risk of producing carcinogens by pyrolytic treatment. By distilling under reduced pressure and by introducing steam it was possible to maintain the temperature of the liquid below 250° C during the distillation. The later stages of sub-fractionation are all being planned so as to avoid high-temperature treatment, and in this way ensure that any compounds which may be isolated are reasonably certainly present in the original crude oil. The first stage of sub-fractionation of selected fractions prepared by the Thornton Research Centre is being carried out under the supervision of Professor A. Morton in the Department of Chemical Engineering of the University of Birmingham. The 15 sub-fractions (5 from each of the three crudes) are undergoing biological test in the Cancer Research Laboratories and are also being subjected to chemical examination by Dr. W. Carruthers working in the Chemistry Department of the University of Glasgow under the general direction of Professor J.W. Cook. This section of the Report is concerned with the work of Dr. Carruthers.

The hypothetical chemical structure of the unknown carcinogen

It has long been known that mineral lubricating oils are liable to contain carcinogenic constituents. This is undoubtedly responsible for the high incidence of skin cancer among cotton spinners, and the carcinogenicity of the lubricating oil used for the mules has been demonstrated repeatedly by experiments on mice, but no pure carcinogenic compound has hitherto been isolated from such a source. A similar carcinogenic industrial material is coal tar, and from this a potent carcinogenic constituent has been isolated and shown to be a fused-ring aromatic hydrocarbon 3:4 - benzpyrene. Other hydrocarbons of comparable potency, belonging to the same class, have been prepared synthetically. Using as a working hypothesis, therefore, the assumption that the active ingredients of mineral oils may be of the same type, attention is being focussed in the first instance on the aromatic fractions of the oils, and particularly on the polycyclic aromatic materials, which have certain characteristic properties by which they may be recognized. This may help to simplify the course of the investigation, but it is recognized that the active constituents of the oils may be of quite other chemical types and the non-aromatic fractions will certainly not be neglected. There is some justification for devoting more attention initially to the polycyclic aromatic constituents of the oils, for not only do the most potent compounds known belong to this class, but it has been our experience that all of the carcinogenic oils which we have examined contain polycyclic aromatic fractions.

Chemical examination of the fractions

Up to the present the chemical examination of the fractions has inevitably lagged far behind the other phases of the work, for not only have the crude oils had to be selected, procured and fractionated, but the carcinogenic potencies of these fractions have had to be evaluated by laborious biological tests extending over more than a year. Consequently it has been possible only recently to make a start on the main chemical task of attempting the isolation of pure constituents of the oils selected for systematic investigation. Before this start became possible attention was devoted to shorter term projects of examining some oil extracts which had already been shown by Dr. Woodhouse to be carcinogenic and also some related materials separated from petroleum oils. A description of the results of this work is included in this Report[8]. It has proved well worthwhile, not so much for the intrinsic value of the results obtained, but chiefly for the experience which has been gained in devising and studying the methods of separation which will be applied in the main task confronting us in this research. Among the methods studied were fractional distillation, chromatography, extraction with concentrated sulphuric acid, and formation and fraction crystallisation of molecular compounds with polynitro compounds such as picric acid and s-trinitrobenzene.

The extracts examined were commercial products obtained by extracting certain petroleum distillates with liquid sulphur dioxide, a solvent which selectively dissolves the aromatic components. The extracts were generally dark viscous oils of high boiling-point, containing only small amounts of material distilling below 200° C/0.2 mm. Higher-boiling material was distilled at temperatures up to 250° C on the mercury pump (10^{-3} - 10^{-5} mm.), affording viscous orange or yellow distillates. Because of the high boiling points fractional distillation could not be employed to achieve an initial sub-division of the extracts. For this purpose use was made of chromatography.

The viscous distillates obtained as above were chromatographed on a large scale on columns containing large amounts of alumina, with the object of obtaining less complex fractions which might serve as the basis for further separations by crystallisation of molecular complexes or other means. By elution with petroleum ether containing increasing proportions of benzene, a large number of arbitrary fractions was collected of increasing aromaticity. These fractions were purely arbitrary; there was no clear demarcation between them. In general, elution with petroleum ether afforded a considerable amount (50-60 per cent of the charge) of almost colourless eluate, representing the least aromatic portion of the fraction. Continued elution with petroleum - benzene then afforded fractions of increasing refractive index and deepening colour, containing more aromatic material. The last eluates were usually dark orange viscous gums. From many of these later fractions highly coloured crystalline picrates could be obtained by treatment with picric acid in alcohol. These were generally dark red or brown in colour. They could be crystallised from alcohol or benzene, but generally were obtained in poor yield, and none has yet been obtained in a pure state. On decomposition only small amounts of oily material were obtained. These picrates must represent a considerable concentration of aromatic material over that present in the original extract, but it is clear that they are still complex mixtures. Only by working on a very large scale will it be possible to obtain sufficiently large amounts to offer any prospect of achieving purification of the picrates by crystallisation or other means.

In some cases the fractions obtained by chromatography were further sub-divided by repeated chromatography on alumina. From one of the extracts treated in this way (Extract 33) four crystalline solids were isolated. These were readily obtained homogeneous by crystallisation. One of them has been identified as the pentacyclic aromatic hydrocarbon perylene. The others have not been completely identified. One appears to be an aromatic hydrocarbon of the formula $C_{24}H_{24}$, $C_{25}H_{24}$, or $C_{25}H_{26}$. The ultra-violet absorption spectrum suggests that it is a derivative of chrysene with a large alkyl substituent of six or seven carbon atoms possibly located at position 3 or 4 of the nucleus. The two other compounds isolated are not aromatic. They have the approximate formulae

8 See below p. 13.

fractions of H showed weak maxima in the region 430-440 mμ, but were otherwise smooth. There was, however, no evident relationship between the spectra and the biological properties of the fractions (see Figs. 3-6).[10]

Biological testing at Birmingham and at London of the five fractions prepared at the Thornton Research Centre from the three crude oils (Kuwait, Lagunillas and Oklahoma) being used for the main systematic investigation has indicated that fraction 4 is the most active in each case (designated K-4, L-4, and O-4 respectively). These are dark coloured fairly mobile oils, boiling in the range 330-400° C, 317-384° C and 296-400° C respectively. These active fractions have been further sub-divided by Professor Morton into four sub-fractions and a residue (designated K.4-1 up to K.4-5 etc.) and these are now being tested biologically. Samples of all of these sub-fractions (except K.4-4 and L.4-1) have been made available to us, and we have carried out some preliminary experiments with them. For purposes of comparison, the ultra-violet absorption spectra of the three main fractions (K.4, L.4, and O.4) and the sub-fractions were measured in cyclohexane solution over the range 240-440 mμ. The three main fractions were very similar and showed only general absorption with a decrease of intensity towards the long wave length. With most of the sub-fractions, however, there were distinct maxima or points of inflection at about 325 mμ and 385 mμ (see Figs. 7, 8, 9). The Oklahoma sub-fractions O.4-1, 2, 3 also showed maxima at about 255 mμ. Some of these maxima may be due to anthracene hydrocarbons. Only the three residues, K.4-5, L.4-5 and O.4-5, and the fractions O.4-4 gave smooth curves similar to those of the main fractions. The similarity of the spectra of the other sub-fractions is noteworthy.

The main fraction K.4 has been examined in more detail. The aromatic components were concentrated by selective absorption on a column of silica gel, and the concentrate distilled and collected in 2-3° fractions. Forty-one fractions were taken. These fractions readily yielded orange-red crystalline picrates with picric acid, some of which were crystallised and analysed, although they were not homogeneous. The analyses indicated that the aromatics present consisted of compounds of the C_{15} to C_{19} range, suggesting three or four rings in their molecules. These picrates gave positive tests for sulphur, but the analysis showed that it was not present in significant amount. The ultra-violet absorption spectra of the oils recovered from these picrates were examined in cyclohexane solution. The oils recovered from the earlier picrates of the earlier fractions of the distillations (b.p. $\leq$ 170°/0.4 mm.) gave spectra resembling each other closely, and showing distinct maxima in the region of 375-380 mμ, and others of less intensity at about 360 mμ and 325 mμ. The spectra of the fractions from which these picrates were obtained were also measured and found to be similar to those of the material recovered from the picrates.

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10. In these Figures and in Figures 7-13 the abscissae represent wavelengths, and the ordinates give a measure of the amount of light absorption at a given wavelength. The absorption is usually designated by the quantity $\log. \varepsilon$ (ε being the molecular extinction coefficient) which is defined by the equation

$$\log. \frac{I_o}{I} = \varepsilon R o$$

Hence $\log. \varepsilon = \log. R - \log. c$ — where

$R = \log. \frac{I_o}{I}$, and c = concentration in gm.-molecules/litre

In the Figures the expression $\log. \varepsilon$ is used for the absorption spectra of pure compounds (Figs. 11 and 12), but in the cases of the oil fractions the molecular weights of the absorbing compounds are unknown and the intensity is shown therefore as $\log. R - \log. c$, where c is the concentration in gm./c.c.

$C_{21}H_{31}O$ and $C_{20}H_{29}O_2$, and are extremely resistant to chemical reagents. The latter compound has been more extensively investigated. What evidence there is of its structure seems to indicate a saturated naphthenic structure containing seven rings. The function of the oxygen atoms is unknown.

There was no absorption of hydrogen over a palladium catalyst and the compound was unchanged after attempted selenium dehydrogenation and after heating under pressure with aqueous or alcoholic hydrochloric acid. Reactions for carbonyl and hydroxyl groups were negative.

We also investigated the possibility of effecting a concentration of some of the aromatic components in the extracts by utilising the selective solvent action of concentrated sulphuric acid. This is known to dissolve certain polycyclic aromatic hydrocarbons (e.g. 3:4 benspyrene) without sulphonation, regenerating them on dilution with water. It was found that generally extraction of a petroleum ether solution of the distillates with concentrated sulphuric acid did indeed lead to the separation of a small proportion of the original fraction, but this was still complex in nature. This was shown by the ultra-violet absorption spectra of fractions prepared in this way. These showed only general absorption and were completely devoid of any characteristic features. The bulk of the oils was completely unattacked by cold concentrated sulphuric acid. Highly coloured crystalline picrates could be readily isolated if these extracts were further broken down by chromatography on alumina and the fractions thus obtained treated with alcoholic picric acid. These picrates were far from homogeneous, and again the amounts obtained were too small to allow of much separation by crystallisation.

Parallel with this work we also examined some samples of oleum sludge and solid sodium sulphonates, supplied to us by Manchester Oil Refinery and the Stanlow refinery of Shell. These are produced in the refinery by treatment with oleum of the undissolved material (the raffinate) from the liquid sulphur dioxide treatment. Any aromatic hydrocarbons present either dissolve in the oleum (partly as sulphonic acids) or form sulphonic acids soluble in the oil. The former solution comprises the oleum sludge, and solid sodium sulphonates are obtained from the oil-soluble sulphonic acids. It seemed possible that these materials might contain some of the polycyclic aromatic hydrocarbons of the original oil and an attempt was made to isolate such compounds from them. The solid sodium sulphonates were hydrolysed with hydrochloric acid under pressure, and the recovered oil distilled and chromatographed. The fractions obtained gave only very pale colourations with picric acid, however, and in no case was a solid complex isolated. Similar results were obtained with the oil recovered from the oleum sludge. It seems probable, therefore, that these materials do not contain any appreciable amount of polycyclic aromatic compounds, and further examination of them was therefore postponed.

The ultra-violet absorption spectra of four carcinogenic extracts supplied by Dr. Woodhouse (F, G, I, K)[9] were measured to see if they contained any common feature which might be related to their biological activity. In this connection it is noteworthy that American workers at the Kettering Laboratory of the University of Cincinnati have reported a relationship between the carcinogenic potencies of certain cracked oils and the intensity of an absorption band at 430 mμ. In the present case the spectra of fractions prepared from the four crude oil extracts by distillation were examined over the range 300-450 mμ in cyclohexane solution. Generally there was a gradual fall of intensity towards the long wavelengths. The spectra of I and K showed no maxima or points of inflection; the higher boiling fractions of F and the

9. For properties and methods of preparation see Section III Tables VII(a) and VII(b).

FIG. 3.

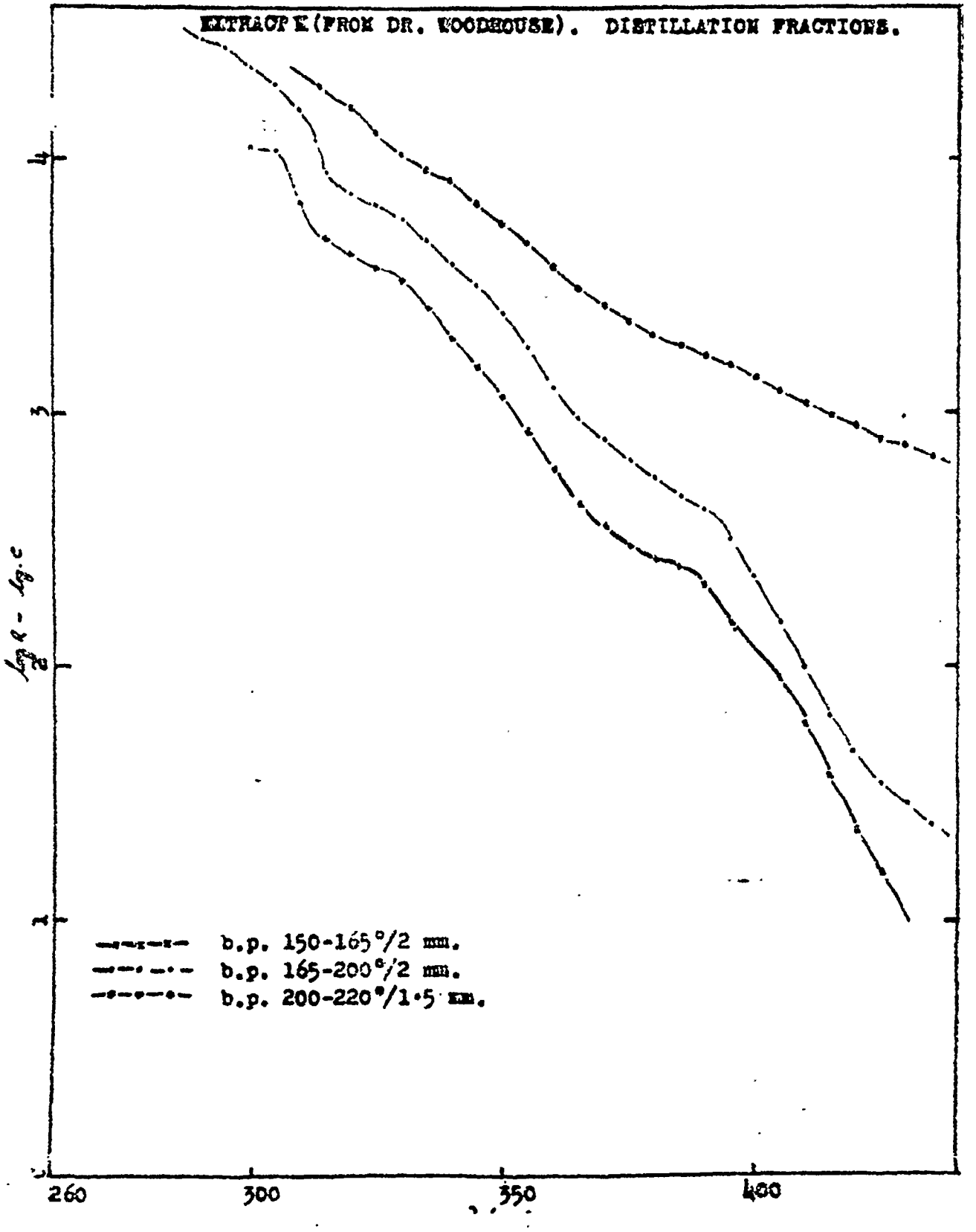

EXTRACT E (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
4
3
2
1
260
300
350
400
b.p. 150-165°/2 mm.
b.p. 165-200°/2 mm.
b.p. 200-220°/1·5 mm.

FIG. 6.

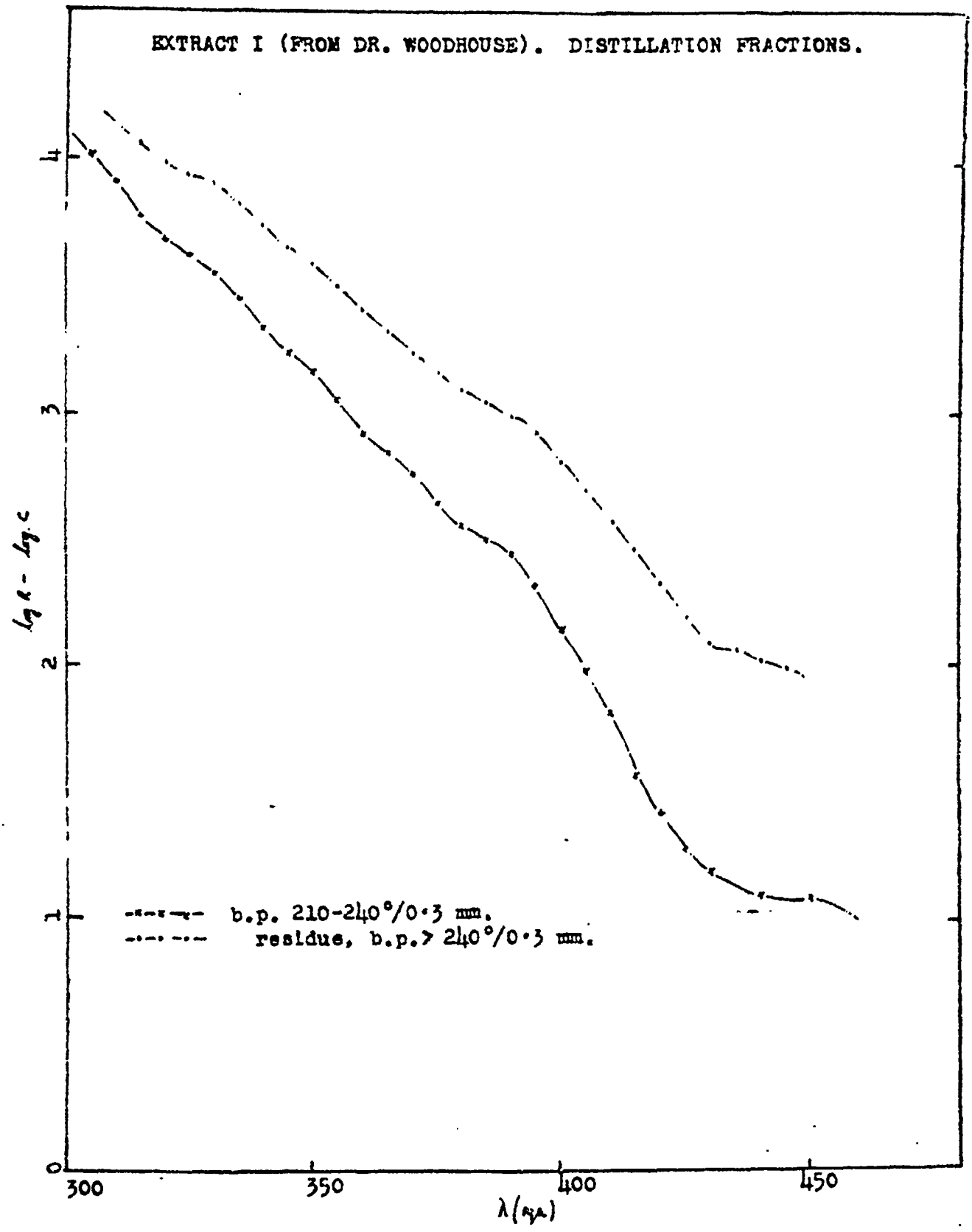

EXTRACT I (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
log a - log c
4
3
2
1
0
300
350
400
450
λ(mμ)
b.p. 210-240°/0·3 mm.
residue, b.p.> 240°/0·3 mm.

FIG. 8.

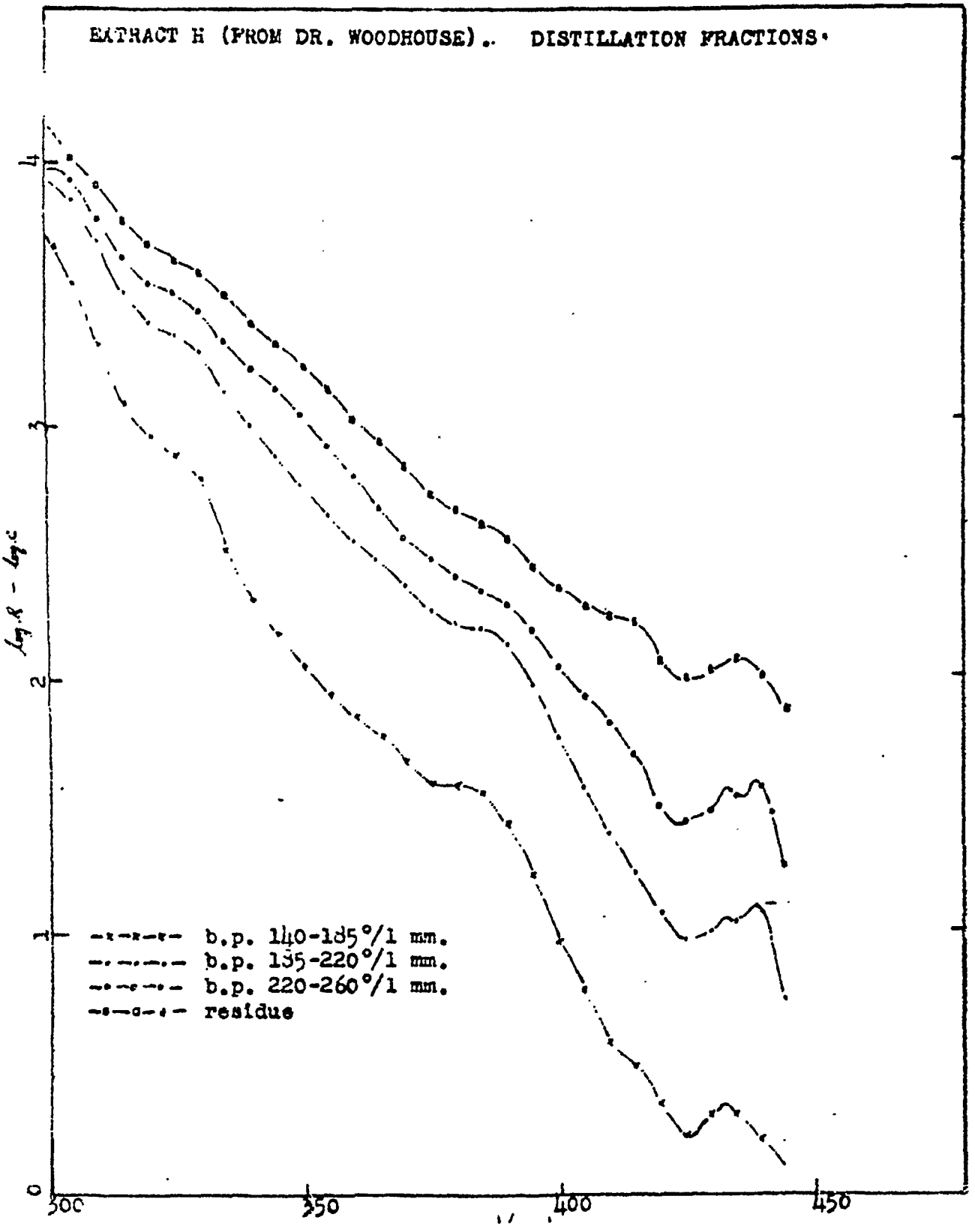

EXTRACT H (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
Log.s - Log.c
4
3
2
1
0
300
350
400
450
b.p. 140-185°/1 mm.
b.p. 185-220°/1 mm.
b.p. 220-260°/1 mm.
residue

FIG. 6.

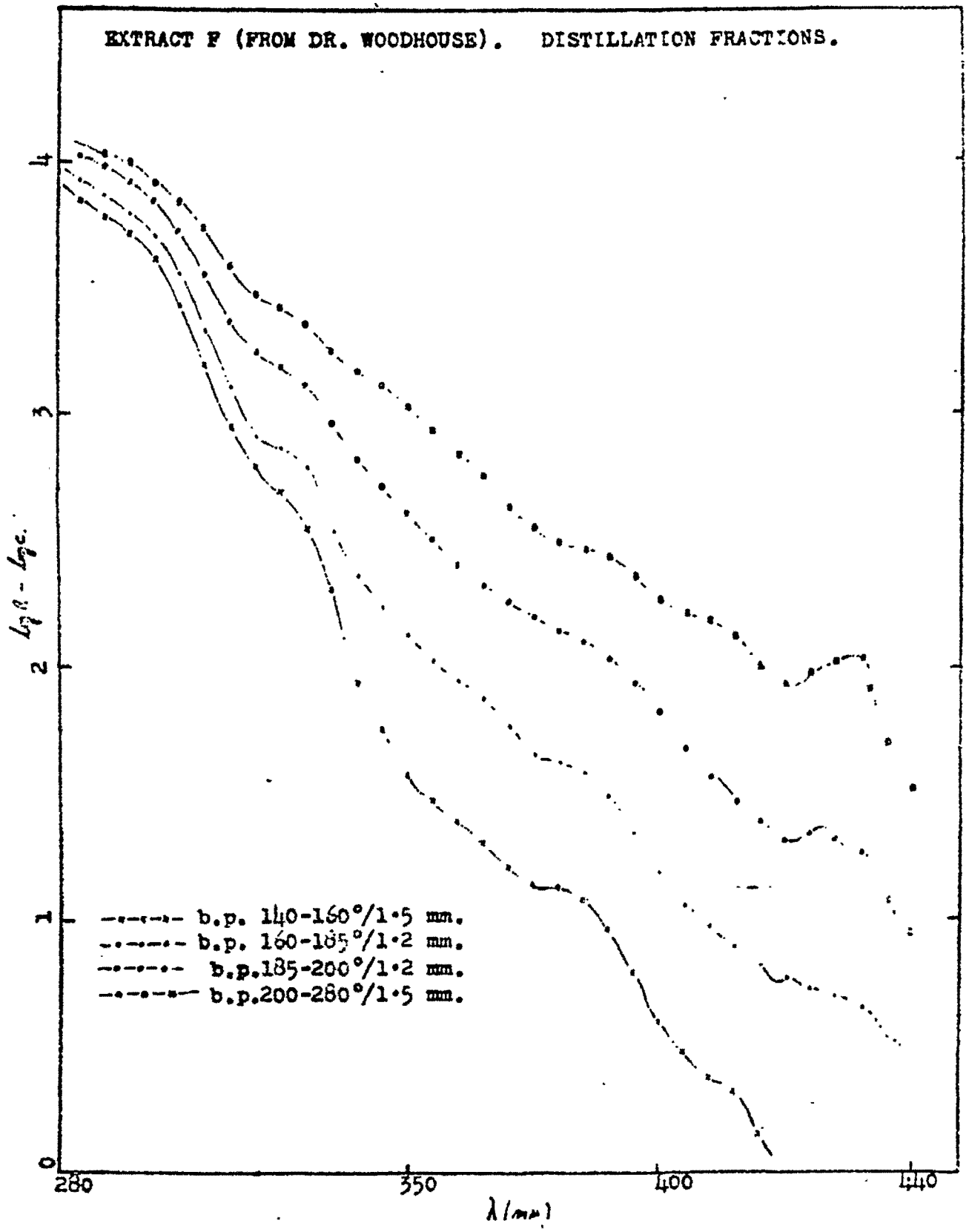

EXTRACT F (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
b.p. 140-160°/1·5 mm.
b.p. 160-185°/1·2 mm.
b.p.185-200°/1·2 mm.
b.p.200-280°/1·5 mm.
0
1
2
3
4
280
350
400
440
λ (mμ)

FIG. 7.

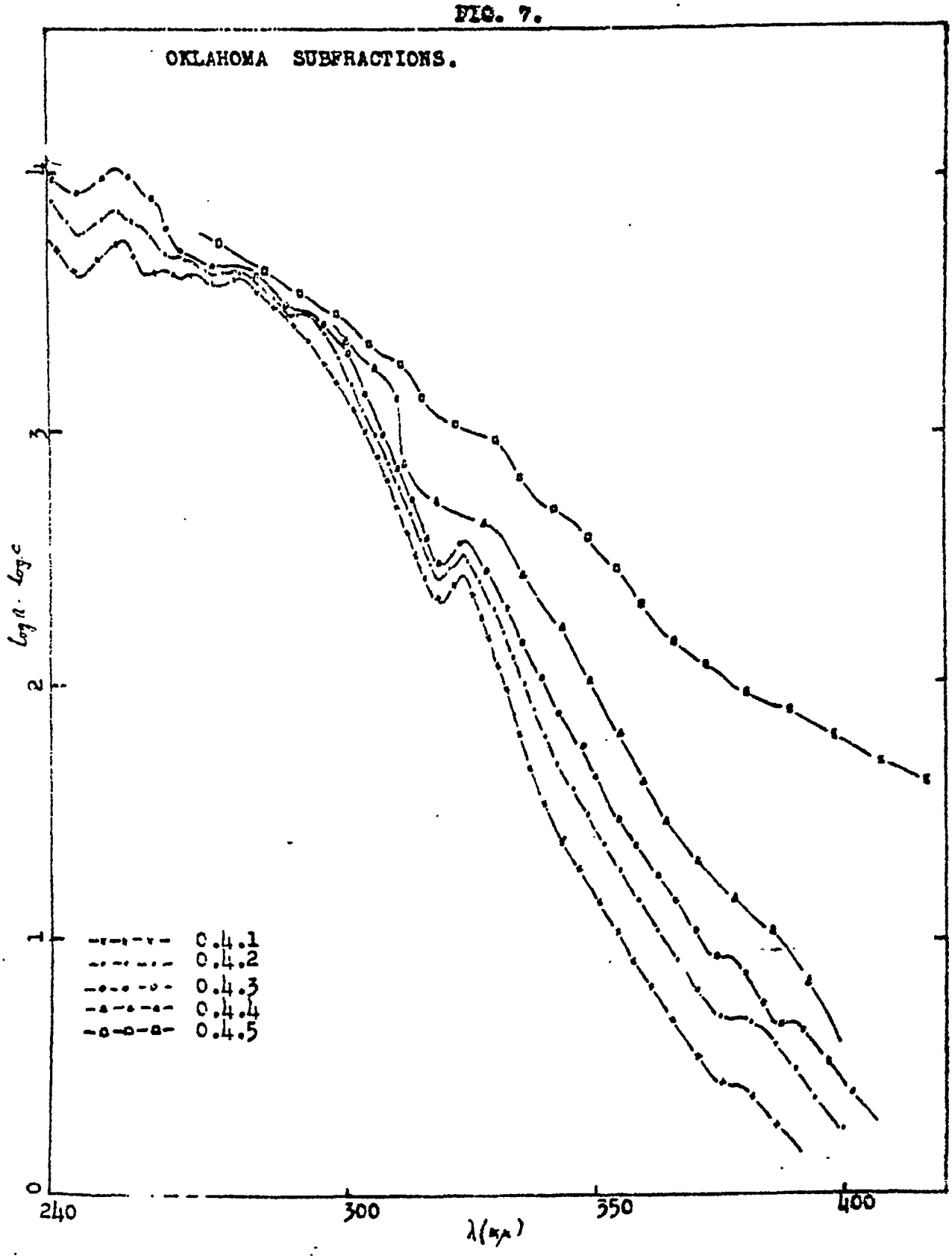

OKLAHOMA SUBFRACTIONS.
Log n · Log c
4
3
2
1
0
240
300
350
400
λ(mμ)
0.4.1
0.4.2
0.4.3
0.4.4
0.4.5

FIG. 8.

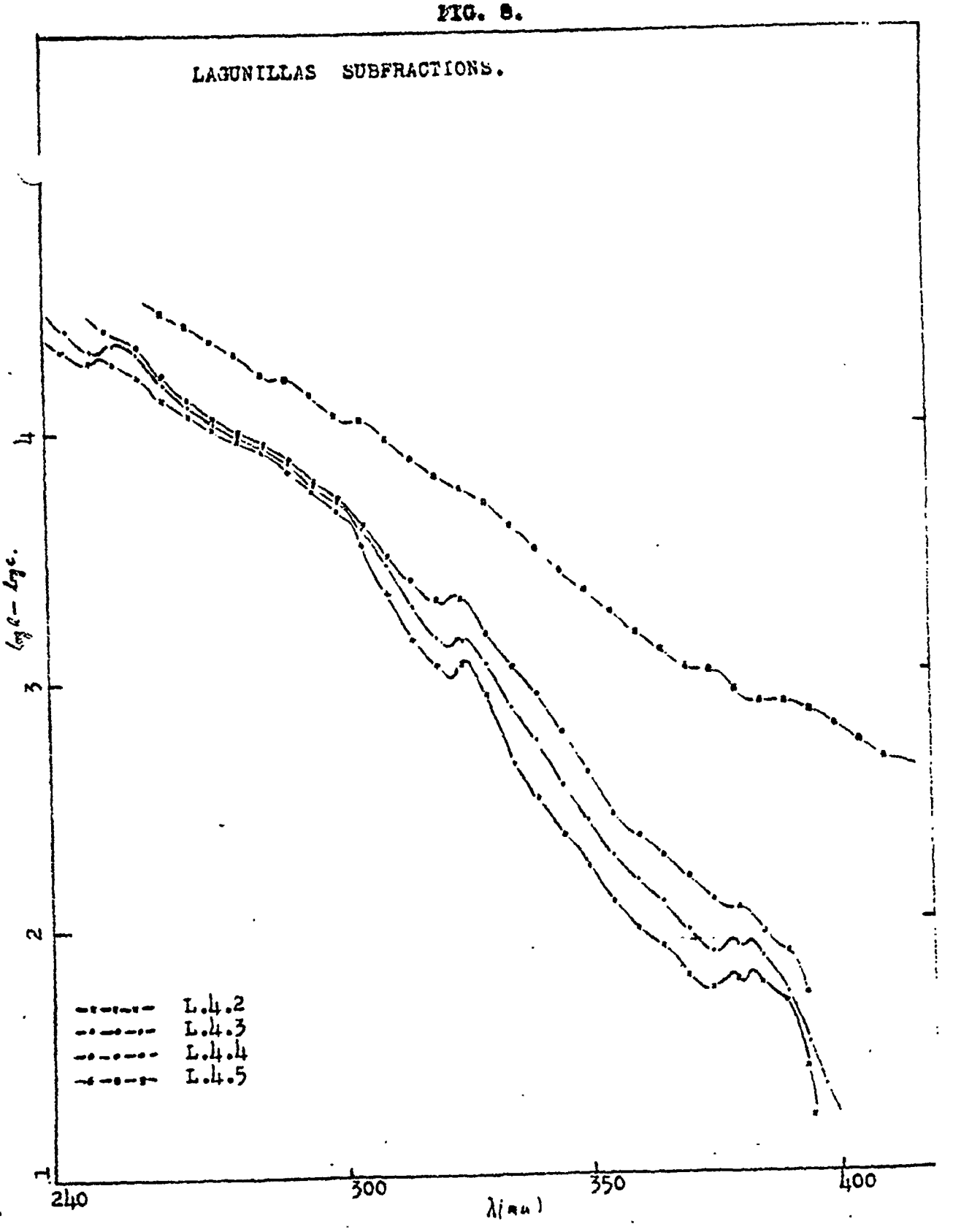

LAGUNILLAS SUBFRACTIONS.
log ε - log c.
4
3
2
1
240
300
350
400
λ (mμ)
L.4.2
L.4.3
L.4.4
L.4.5

FIG. 6.

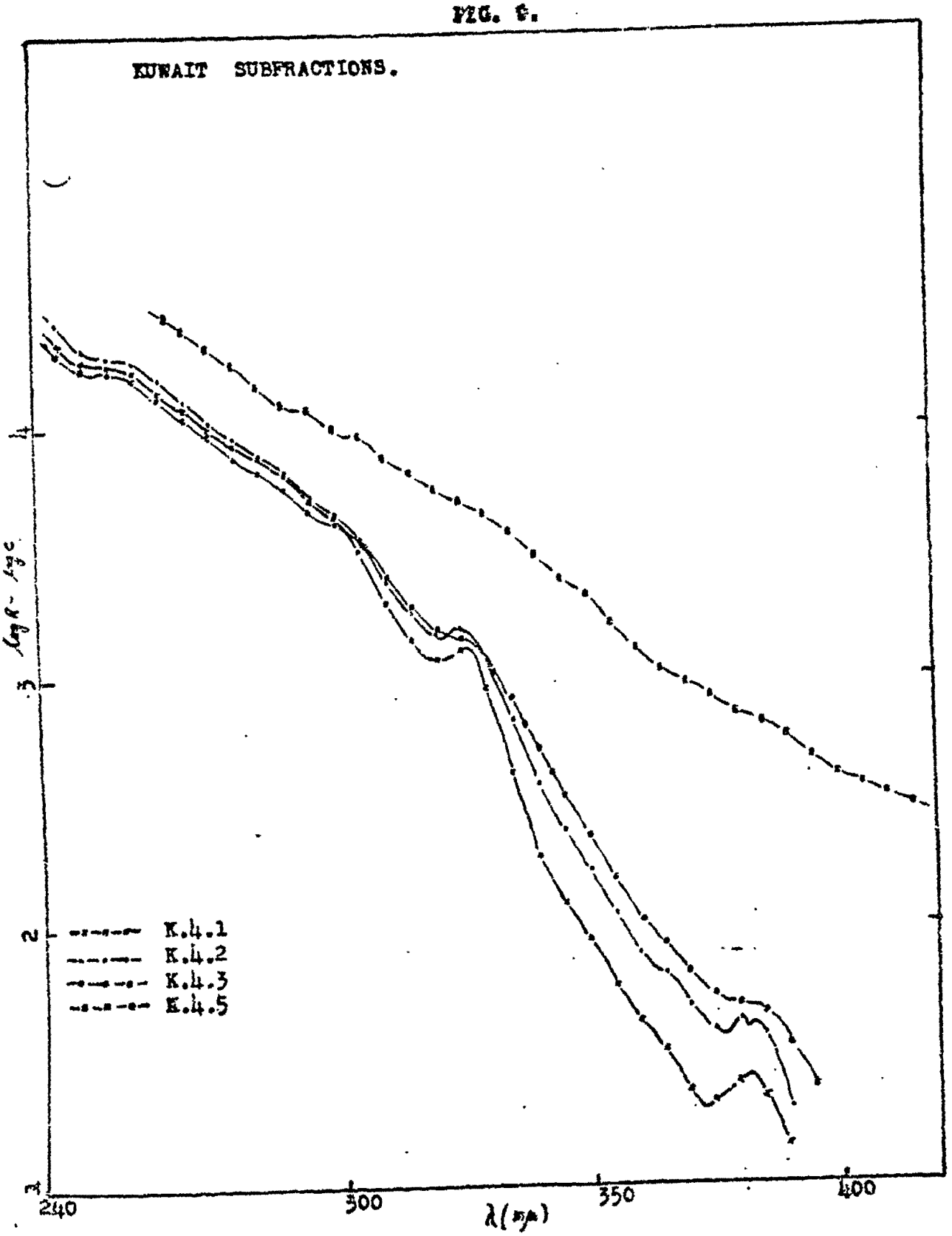

KUWAIT SUBFRACTIONS.
Log ε - Log c
4
3
2
1
K.4.1
K.4.2
K.4.3
K.4.5
240
300
350
400
λ(mμ)

FIG. 1a.

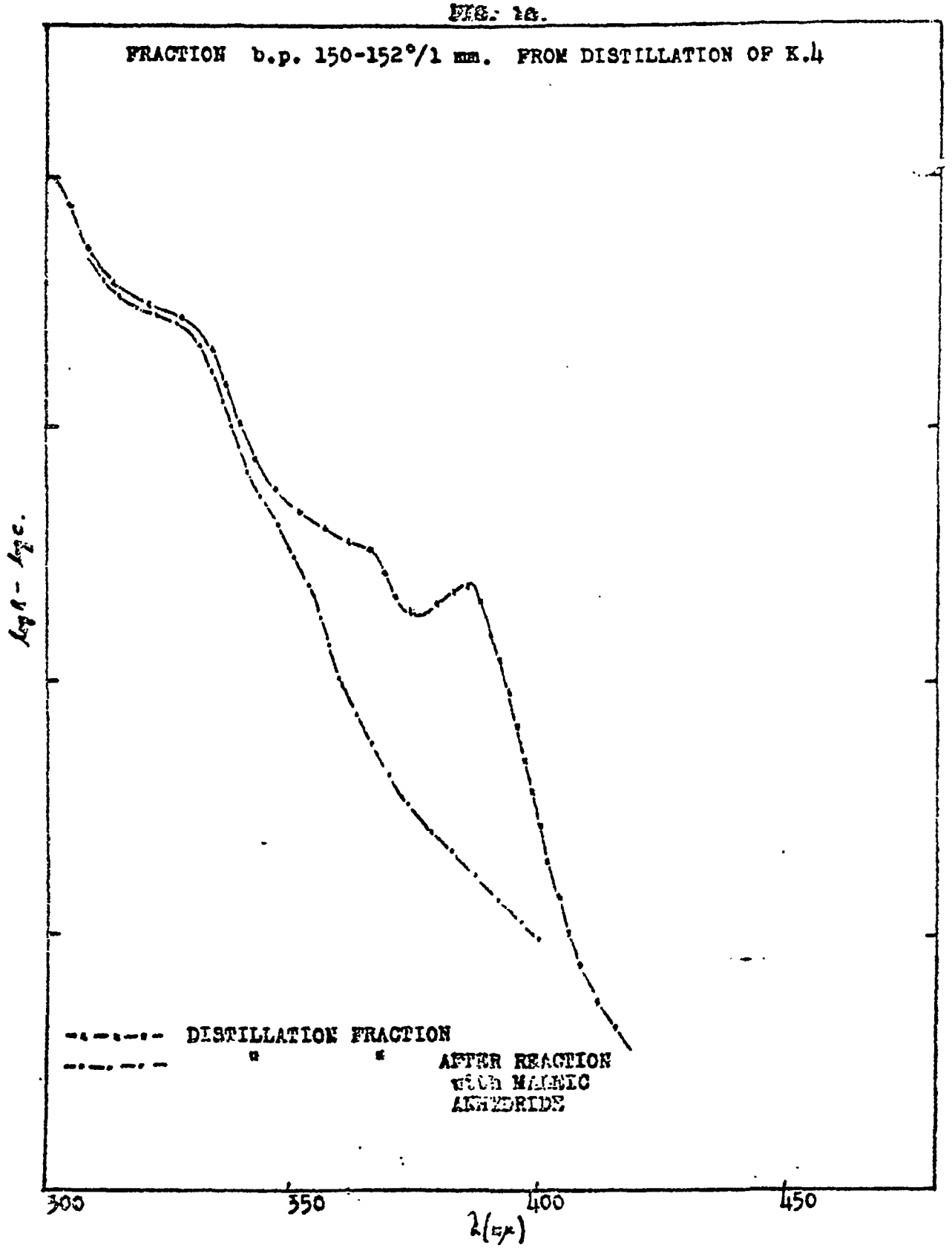

FRACTION b.p. 150-152°/1 mm. FROM DISTILLATION OF K.4
log ε - log c.
DISTILLATION FRACTION
" "
AFTER REACTION WITH MALEIC ANHYDRIDE
300
350
400
450
λ(mμ)

FIG. 11.

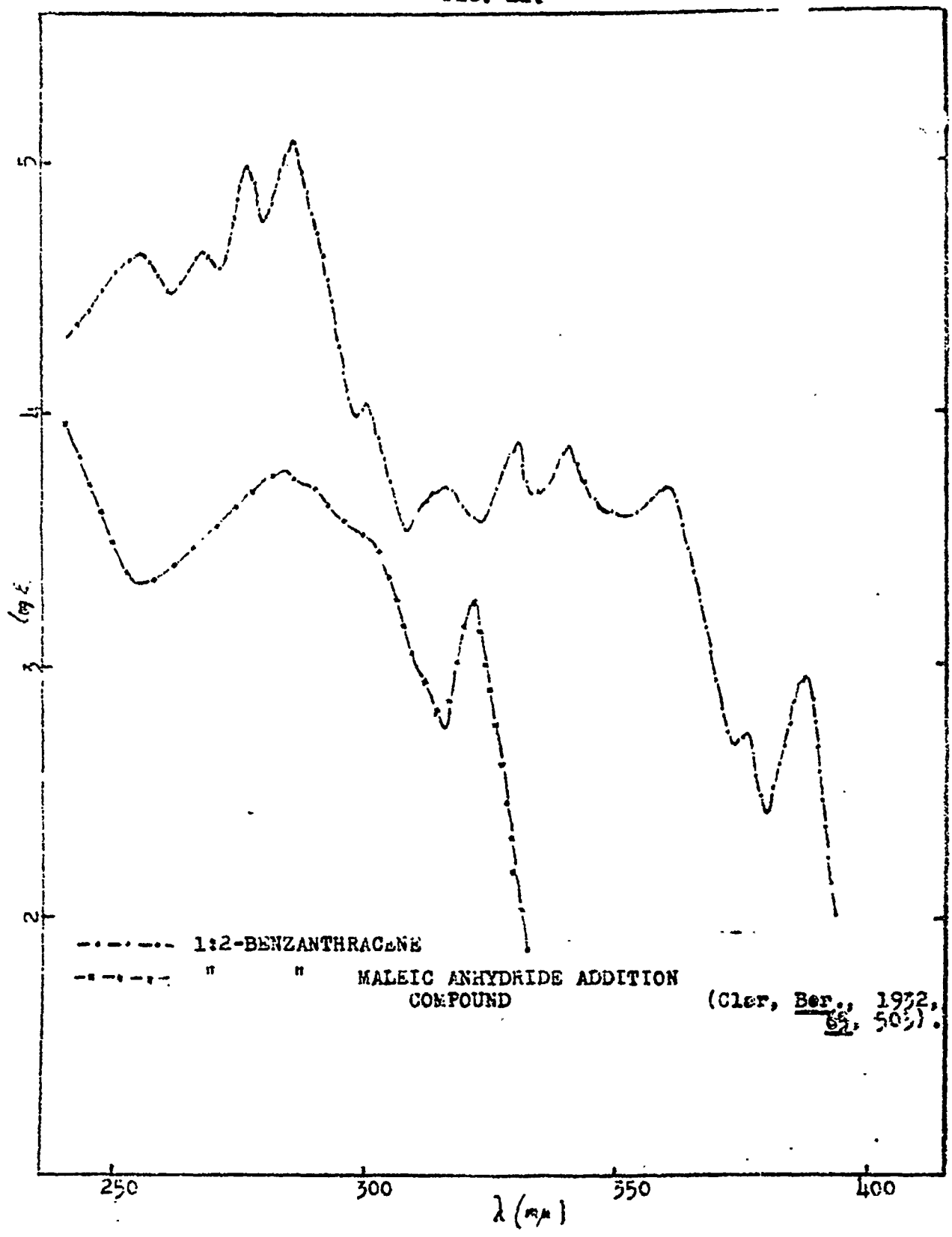

5
4
3
2
log ε
250
300
350
400
λ (mμ)
1:2-BENZANTHRACENE
" " MALEIC ANHYDRIDE ADDITION COMPOUND
(Cler, Ber., 1932, 65, 50).

FIG. 12.

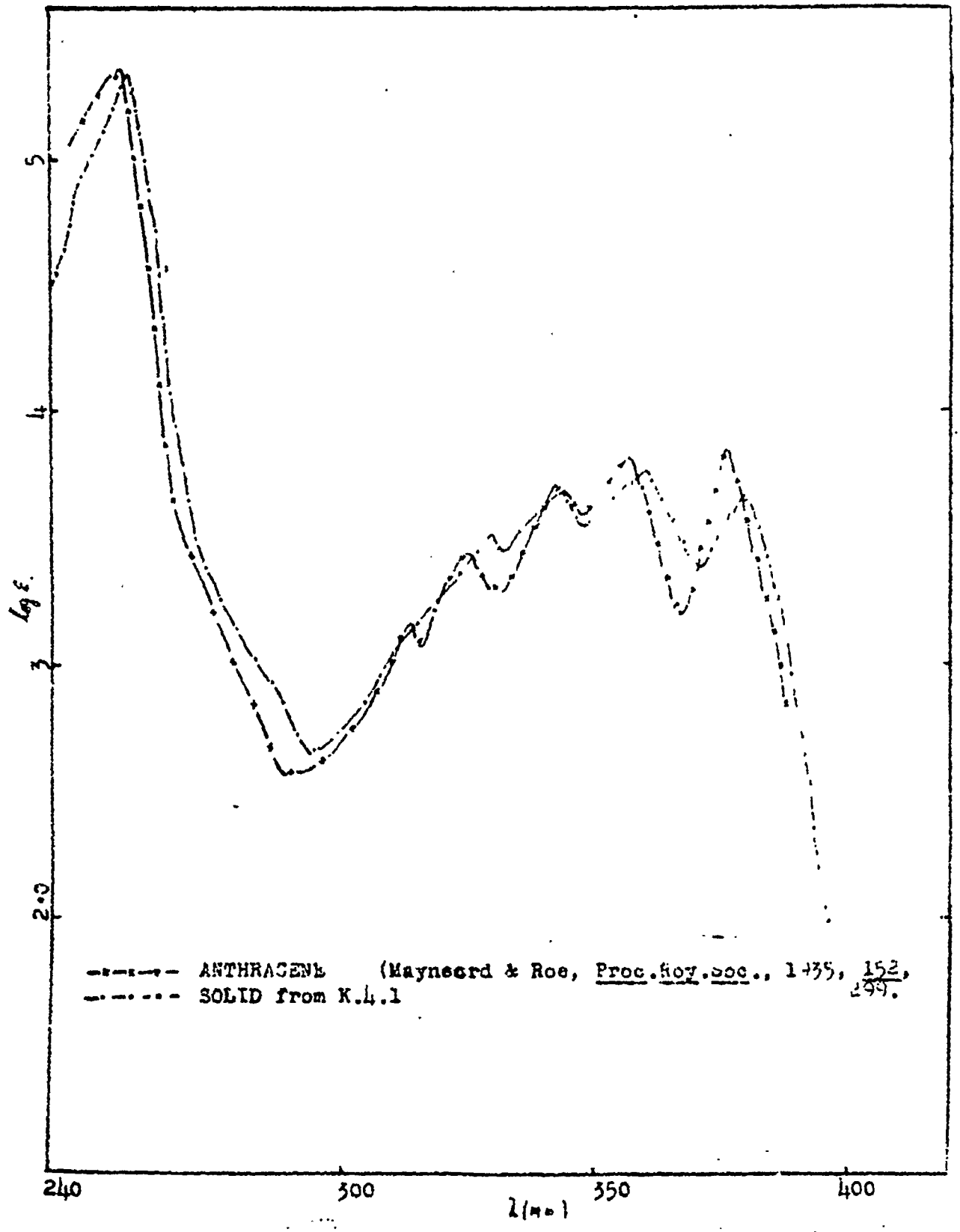
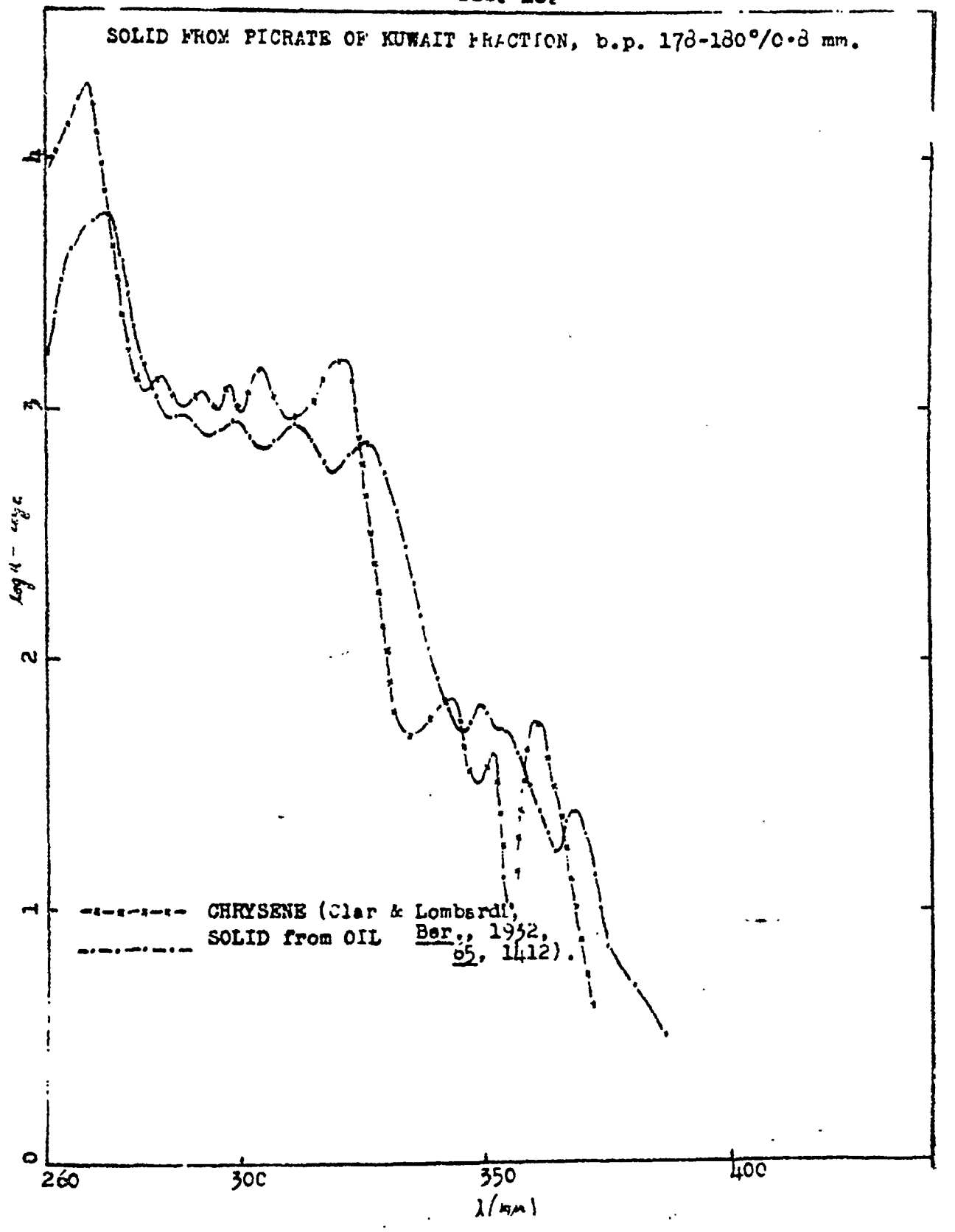

5
4
3
2.0
log ε.
240
300
350
400
λ(mμ)
ANTHRACENE (Maynecrd & Roe, Proc.Roy.Soc., 1935, 152, 299.
SOLID from K.4.1

FIG. 13.


SOLID FROM PICRATE OF KUWAIT FRACTION, b.p. 178-180°/0·8 mm.
4
3
2
1
0
260
300
350
400
λ/mμ
CHRYSENE (Clar & Lombardi, Ber., 1952, 85, 1412).
SOLID from OIL

The spectra are consistent with the presence of anthracene hydrocarbons. This view is supported by the fact that there is a radical alteration of the spectrum after treatment of the oil with maleic anhydride; the bands at 375-380 mμ and 360 mμ are completely eliminated (cf. Fig. 10). (Figs. 10 and 11 give the absorption spectrum of 1:2 benzanthracene and its maleic anhydride addition compound. It will be noted that formation of the latter is attended by a complete change in the character of the spectrum.) The presence of anthracene hydrocarbons in K.4 has now been confirmed by the isolation from the fractions K.4-1, -2, and-3 (see above), through maleic anhydride adducts, of small amounts of crystalline materials, the spectra of which show clearly that they are derivatives of anthracene (Fig. 12). The spectra of the higher boiling fractions from the distillation (b.p. 170°/0.4 mm.) - and of the material recovered from the corresponding picrates - showed more general absorption, with no maxima or pronounced points of inflection. By chromatography of the oil from the picrate of one of these fractions, a solid was isolated whose spectrum (after crystallisation) is clearly that of a chrysene derivative (Fig. 13). These results seem to indicate the presence of three-and four-ring aromatics in K.4, and this is consistent with the boiling point, 330-400° C of the fraction. Anthracene boils at 340° C, pyrene at 404° C and chrysene at 436° C.

Before the results of the biological tests on the main fractions became available, some preliminary work was carried out with the residues from the initial distillation of the three crudes (i.e. K.5, L.5, and C.5). These were black viscous oils, and contained much high boiling material, only a very small amount distilling below 200° C/10^{-5}., affording viscous orange distillates. Fractional distillation was therefore out of the question. As in the case of the extracts already studied, use was made of chromatography to achieve some initial fractionation of these materials. It was found an advantage first to separate the aromatic compounds from the naphthenes and paraffins also present. For large scale work this was best accomplished by extraction of the distillates with furfural, a process used industrially for this purpose. The aromatic extracts thus obtained were chromatographed on alumina as described before. The results obtained were similar to those in the experiments with the extracts. Many of the fractions readily yielded highly coloured crystalline picrates, but in amounts too small to allow purification by crystallisation. The dark red or brown colour of these picrates suggests their formation from complex aromatic material. One deep red picrate from C.5 analysed for a hydrocarbon of approximate formula $C_{26}H_{18}$. Work with these residues was discontinued when the biological tests showed them to be without activity.

Summary

Taken as a whole, the results obtained indicate that it should be possible eventually to separate and identify a number of the polycyclic aromatic compounds present in the actively carcinogenic fractions of the oils. It is clear, however, that this will be a difficult and laborious task and a rapid conclusion of the investigation is not to be expected. This is especially so as the successive stages in the chemical separation of the consitiuents of the fractions will need to be controlled by very time-consuming biological tests. There is always the possibility, however, that the work may be accelerated by the isolation from some of the sub-fractions at present under examination of known carcinogenic hydrocarbons or closely related compounds.

Table VIIa. Birmingham Tests. Data relating to source and characteristics of fractions A - P

Sample	Petrol. Board Designation	Feed stock	Process	Refinery
A	Group 5(71)	Colon Residue (V)	Duosol (Propane/Selecto)	Stanlow
B	Group 5(78)	Eakring Residue	" " "	Stanlow
C	Group 1(72)	Leguinillas L.O.D. (V)	Edeleanu (Duosol/SO_2)	Stanlow
D	Group 1(85)	Concepcion L.O.D. (V)	Furfurel Extract	Shell Haven
E	Group 5A(S.5)	Distn. residue from D. Concepcion L.O.D. (V)	Furfurel Extract	Shell Haven
F	Group 1(11)	W. Beaumont L.O.D. (T)	Edeleanu (Benzol/SO_2)	M.O.R.
G	Group 3(33)	W. Beaumont L.O.D. (T)	" " "	M.O.R.
H	Group 1A(140)	W. Beaumont L.O.D. (T)	" " " (Reprocessed at Lobitos Oilfields Ltd. Ellesmere Port)	Lobitos
I	Group 2(200X)	Eakring L.O.D.	Edeleanu (Benzol/SO_2)	Lobitos
J	Group 4(2228)	Iranian L.O.D.	" " "	Llandarcy
K	Group 1(2241)	Colombian L.O.D.	" " "	Llandarcy
L	Group 4(2252)	Colombian L.O.D.	" " "	Llandarcy
M	Group 5A(2345)	Colombian L.O.D.	" " " (sulphur hardened)	Llandarcy
N	Group 5A(2345)	Colombian L.O.D.	Edeleanu (Benzol/SO_2) (Air blown)	Llandarcy
O	Group 1A(2419)	Tia Juana L.O.D. (V)	Edeleanu Benzol/SO_2	Llandarcy
P	Group 2(200X)	Ecuadorain L.O.D.	" " "	Lobitos

L.O.D. = Lubricating Oil Distillate; V = Venezuela; T = Texas; M.O.R. = Manchester Oil Refinery

Table VIIb. Birmingham Tests. Data relating to characteristics of fractions A - P

Sample	Character of Fraction	How applied	Toxicity by Survival Rate	Viscosity @ 140°F	Redwood 1 @ 200°F	Softening Point (R & B)	Penetration @ 25°C
A	Shiny black tar. Almost solid at 18°C	Solution of 50 g. in 30 ml. toluene	Moderate	-	-	51°C	65
B	Similar to A but slightly more liquid	Ditto	Slight	-	-	37°C	188
C	Very viscous oil Yellow-green fluor-escence	With 10% toluene added	Pronounced	662	-	-	-
D	Similar to C but slightly more liquid	Ditto	Pronounced	473	-	-	-
E	Black pitch	Soln. of 50 g. in 30 ml. toluene	Slight	-	-	37.5°C	160
F	Fairly thin brownish oily liquid	Without diluent	Very toxic	48	-	-	-
G	Very viscous dark brown terry liquid	With 10% toluene	Moderate	-	450	-	-
H	Amber oil. Green fluor-escence	Without diluent	Moderate	140	-	-	-
I	Thin, dark brown tar	Ditto	Moderate	-	236	-	-
J	Viscous brown tar	Soln. of 50 g. in 30 ml. toluene	Moderate	-	824	-	-
K	Thick brown oil	With 10% toluene	Very pro-nounced	190	57	-	-

Table VIIb continued

Sample	Character of Fraction	How applied	Toxicity by Survival Rate	Viscosity @ 140°F	Redwood 1 & 200°F	Softening Point (R & B)	Penetration @ 25°C
L	Very viscous brown tar	With 10% toluene	Pronounced	-	744	-	-
M	Solid pitch, black	50% wt./vol. "suspension" in toluene	Slight	-	-	8[illegible]°C	-
N	Solid black pitch	Ditto	Very slight	-	-	80°C	-
O	Yellow oil marked green fluorescence	Without diluent	Slight	300	-	-	-
P	Very viscous brown tar	With 10% toluene	Fairly great	-	200	-	-

Table VIIc. Birmingham Tests. Carcinogenic response to samples A - P and controls

Material	Estimate of Carcinogenic Response
Extract A	15
Extract B	0
Extract C	20
Extract D	slightly positive
Extract E	0
Extract F	25
Extract G	0
Extract H	20
Extract I	25
Extract J	10
Extract K	30
Extract L	5
Extract M	0
Extract N	0
Extract O	10
Extract P	3
Creosote	50
Anthracene oil	30
Linseed oil	0
Pine Tar oil	0
Butyl phthalate	0
Tricresyl phosphate	-

This estimate is based on the total number of tumours compared with numbers previously obtained with 0.5% solution of 3:4-benzpyrene in benzene. This would be assigned a figure of 60.

Table VIII. Birmingham Tests. Spindle Oils

Spindle Oil	Animals Surviving 25 weeks	Tumours			Skin Reaction	Estimate of Carcinogenic Response
		Papillomata	Carcinomata	Total		
R	19	3	3	6	Slight roughness with epilation	15
S	21	7	2	9	Slight roughness with epilation	25
T	25	2	2	4	Slight roughness with epilation	10
U	16	3	3	6	Rough & dry with epilation	15
V	15	1	2	3	Slight epilation	5
W	19	1	0	1	Smooth & dry with epilation	5
X	25	1	0	1	No epilation	5

Table VIIIa. Birmingham Tests. Other control oils

Anthracene oil	20	8	6	14		30
Creosote oil	19	10	9	19		50
Pine oil	18	0	0	0		0
Linseed oil	44	0	0	0		0

Table IX. Birmingham:- "Duplicate Experiments"

1st Experiment				
Fraction	Mice Surviving 25 weeks*	Papillomata	Carcinomata	Total tumours
B	32	1	1	2
D	15	2	1	3
E	35	2	0	2
F	12/62	2	4	6
G	23	2	0	2
H	26	3	4	7
I	29	2	6	8
K	20/70	4	4	8
L	30/60	2	1	3
M	30	0	0	0
2nd Experiment				
Fraction	Mice Surviving	Papillomata	Carcinomata	Total tumours
B	22	2	1	3
D	12	1	3	4
E	22	1	2	3
F	15	5	3	8
G	12	2	0	2
H	16	3	5	8
I	15	5	3	8
K	20/60	5	3	8
L	20	3	1	4
M	20	1	0	1

* Survivors out of 50 unless otherwise stated

Section III

THE RESULTS OF BIOLOGICAL TESTS

The technique employed in the biological tests on behalf of the Medical Research Council were based to a considerable extent on the experience gained during work previously carried out for the Petroleum Board. A part of the work during the period under review dealt with extracts utilised in the earlier work. A short outline of this will therefore be given.

Tests of mineral oil fractions carried out at Birmingham for the Petroleum Board

A. Birmingham. Tests on 16 fractions and residues. During 1944-1945 a series of 16 fractions, A-P, were tested on groups of 50 mice. These consisted of lube distillates and residues from crude oils originating in U.S.A., Venezuela, Ecuador, Colombia, Iran and U.K., processed by Duosol, Edeleanu and Furfural methods. Their action on animals, including the production of benign and malignant tumours, was noted and an estimate of their relative carcinogenic potency was made.

During the same period 6 materials, not of mineral oil origin, were tested either as controls or on account of their use industrially. These were Creosote oil, Anthracene oil, Linseed oil, Pine Tar oil, Butyl phthalate, and Tricresyl phosphate. (The last was so toxic that long-term experiments with it were abandoned.)

The creosote and anthracene oils were potent carcinogens. The 'vegetable' oils did not produce any tumours. The characteristics of the materials and a summary of the results of these tests are given in TablesVII(a) and VII(b).

B. Birmingham. Tests on 'Spindle' oils and 'White' oils. During 1946-1947, using similar techniques, the carcinogenicity of 7 spindle oils designated R, S, T, U, V, W, X, and 3 samples of 'white' oils designated A, B, P1, were tested. (These symbols have no connexion with the mineral oils mentioned in A.) The spindle oils in every instance had some carcinogenic activity and the general results are given in Table VIII.

The 'white' oils, however, caused no skin reaction, no epilation and no tumours on the mice during the painting and observation period of 50 weeks or more. The complete absence of any reaction indicated that such substances might be employed safely in industry and also in subsequent animal tests as a solvent for standard carcinogens.

C. Birmingham. Duplicate experiments. During the same period (1946-1947) a repetition with 10 fractions selected from those tested during 1944-1945 was undertaken in order to ascertain how close the results would be to one another and what degree of uncertainty might be introduced by unavoidable variations in technique under such circumstances.

The results of these tests were analysed statistically by Irwin and the findings were published (Woodhouse & Irwin, 1950)[11]. The following summary of technique, observations and results are taken from the published paper. Subsequently some slight modifications in technique were introduced following discussion with Professor Hadden, Dr. Carr and Dr. Hieger, so that tests which were to be carried out in collaboration with

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11. Woodhouse, D. L. & Irwin, J. O., (1950). J. Hyg., Camb. 47, 121.

the Chester Beatty Research Institute should be made as far as possible under standard conditions. These changes are noted below.

General arrangements for the tests

Animals

Each fraction was tested on 50 albino mice obtained from one source. They were approximately 10 weeks old when the experiments commenced and 15-20 g. in weight; males and females were used in about equal numbers for each fraction. They were housed in wooden boxes 10" x 4½" x 4½", two animals per box, bedded with sawdust and 'cellulose wool'. A diet of wholemeal bread and rusks with water and fresh milk was given daily, with the addition of crushed oats and a little bran two or three times a week.

Experimental technique

The oils were taken from bottles by means of short glass rods and applied to the skin in the interscapular area from which the hair was removed with dilute sodium sulphide some days before commencing the paintings. An area about 1½ cm. in diameter was covered, and the amount of oil used was kept as constant as possible, though it was difficult to do this exactly with samples of widely different viscosity. With few exceptions, applications were made twice weekly for a period of 25 weeks; on the death of any animal which had survived a period of more than 12 weeks the skin of the treated area was removed for microscopic examination.

It was thought desirable to use the substances neat whenever possible, but in some instances they were so viscous that it was necessary to add a 'solvent'. Acetone has been favoured by many investigators for making solutions of carcinogenic hydrocarbons, but some of the specimens were not sufficiently miscible in this medium; benzene or toluene, however, yielded homogeneous solutions. It is known that both of these solvents are somewhat toxic, but the latter has been shown to be less injurious to animals. The amount added to individual fractions is given in Table 7b. This also described the general characters of the fractions and their general effects on the mice during the experiments.

Results

The number of animals surviving 25 weeks or more and the total number of tumours produced by the various fractions in the experiments are given in Table IX. Fuller analysis of the results by statistical methods are given in the paper cited above. Two illustrative examples of data from which these statistical calculations were made are also set out in Tables X and XI:- (for Expt. 1, fraction K, a moderately carcinogenic sample and Expt. 1, fraction L, a weak carcinogen. The complete data are available.)

Conclusions

From a general study of the data in Table IX and the 'life sheets', of which Tables X and XI are examples, it is possible to draw some general conclusions.

1. There was a fair agreement in the results of the two series, though some distinct differences may be noted.

2. On the whole, the survival rate in Experiment 2 was consistently less than in Experiment 1.

3. There appeared to be a slightly greater tumour response in a number of tests in Experiment 2.

4. Several fractions had a very similar, moderate degree of activity (F, H, I, K,), while fraction M was very weak or of negligible activity.

Table X. Birmingham Duplicate Experiments.
Mice Life Sheet

Fraction X - Experiment 1.

Weeks from Start of Experiment	Survivors (out of 50)	Survivors with tumours	% Survivors with tumours	New tumours in previous week
16	39	0	0	0
17	38	0	0	0
18	36	0	0	0
19	36	0	0	0
20	34	2	5.9	0
21	31	3	9.7	2
22	29	4	13.8	1
23	29	4	13.8	1
24	28	5	1718	0
25	26	6	23.1	1
26	26	6	23.1	1
27	25	7	28.0	0
28	25	7	28.0	1
29	25	7	28.0	0
30	25	7	28.0	0
31	25	7	28.0	0
32	25	7	28.0	0
33	24	7	29.2	0
34	23	7	30.4	0
35	23	7	30.4	0
36	23	7	30.4	0
37	23	7	30.4	0
38	23	7	30.4	0
39	23	7	30.4	0
40	23	6	26.1	0
41	22	6	27.3	0
42	21	5	23.8	0
43	20	5	25.0	0
44	18	5	27.8	0
45	17	4	23.5	0
46	15	4	26.6	0
47	14	3	21.4	0
48	13	3	23.1	0
49	12	2	16.6	0
50	11	2	18.2	0

Table XI. Birmingham Duplicate Experiments.
Mice Life Sheet

Fraction L - Experiment 1

Weeks from Start of Experiment	Survivors (out of 60)	Survivors with tumours	% Survivors with tumours	New tumours in previous week
30	30	0	0	0
31	30	0	0	0
32	30	0	0	0
33	28	0	0	0
34	28	1	3.6	0
35	28	1	3.6	1
36	28	1	3.6	0
37	26	1	3.8	0
38	25	2	8.0	0
39	25	2	8.0	1
40	24	3	12.5	0
41	22	3	13.6	1
42	19	3	15.8	0
43	17	3	17.7	0
44	17	3	17.7	0
45	17	3	17.7	0
46	14	3	21.4	0
47	13	3	23.1	0
48	12	2	16.6	0
49	9	1	11.1	0
50	6	1	16.6	0

5. The investigation indicated that in order to obtain a general estimate of the carcinogenic properties of this type of material at least 50 animals should be employed.

For reliable comparison of certain carcinogenic potencies, it is desirable that fractions should always be compared concurrently in an experiment carried out on randomised batches of mice. The paintings in all the tests necessary on any one day should be carried out by a single individual. Under these conditions it appears possible to achieve reproducible results.

Summary of Irwin's findings

Woodhouse's experiments on the carcinogenicity of 10 different fractions for the skin of mice have been analysed statistically. Two experiments separated by a considerable period of time were performed on each fraction. A number of different measures of carcinogenicity were used. All agree in showing that:

1. The apparent carcinogenicity was greater in the second series of experiments.

2. There were significant differences of carcinogenicity. The fractions can be divided into three groups within which the carcinogenicities were about the same, F, H, I, K; B, D, C, L, E; M. The groups are in order of decreasing activity.

D. Research co-ordinated by the Council's Committee on the Carcinogenic Activity of Mineral Oils

Period 1949-1950 - 'Dilution experiments'

During this period work for the development of a standard method of expressing carcinogenic potency was carried out and, following on the observations of Professor Squire and Dr. Cruickshank and the advice of Dr. Berenblum, experiments to test the suitability of rabbits for assays of carcinogenicity were also conducted. The mouse dilution experiments were carried out in duplicate, at Birmingham and at the Chester Beatty Research Institute. The rabbit dilution experiments were carried out at Birmingham only.

The objects in these tests were:-

1. To ascertain the effect of diluting the material to be tested on the carcinogenic response of the skin of mice, with a view to providing data which will serve as a basis for future tests on fractions or isolated compounds separated from the crudes or distillates by physical or chemical methods.

2. To compare the effects of the same dilutions of these materials on the response of rabbits when they are applied at a number of sites on each animal.

Standardised technique agreed upon by the workers at Birmingham and London in the light of the experiments outlined above and the design for the rabbit tests devised by Irwin were followed. The chief points respecting the experiments are as follows.

Mice

White albino mice, strain Clarke O.1, 12 weeks old at the start were used, 25 males and 25 females on each fraction. The sexes were separated and the animals housed in metal boxes 5 in a box. Food for the mice consisted of proprietary cubes with supplement of cod-liver oil (on bread), milk, etc., with water ad libitum. The applications of

the samples were made twice weekly by glass rod applicators, to the areas from which hair had been removed by clipping. The results are given in Tables XIIIa-g, XIIIa-e XIV, XV and have been analysed statistically by Irwin.

Rabbits

Short haired males of 'Old English' type with erect ears chiefly white with some coloured patches, 4 months old at the start were used. They were housed in separate cages with a painting chart for each animal. Food consisted of oats and bran with a small amount of cabbage or roots. Applications to the designated sites were made after hair had been removed by clipping.

All deaths, papillomata etc. were recorded to the nearest half week. The mice experiments commenced on 14th March, 1949, the rabbit experiment on 12th May, 1949 and both series were continued for 12 months.

The following materials were used in these tests:-

1. 9:10-dimethyl-1:2-benzanthracene in dilutions of 0.1% (D/1)[12], 0.025% (D/4); 0.00625% (D/16); 0.00156% (D/64).

2. Oil fraction I, neat; diluted 1/4; diluted 1/16; diluted 1/64.

3. Oil fraction F; diluted as for I.

The solvent diluting medium was colourless B.P. grade of liquid paraffin. F and I were fractions similar to those used in the experiments described above and had been proved to produce epitheliomata in mice. The fractions and solutions were stored in amber coloured bottles and in all the experiments recorded in this report precautions were taken to avoid exposure to light or any accidental contamination.

36 rabbits were used, divided into 12 randomised groups of 3. Each rabbit was painted on 6 sites:- I, II, - both ears, outer surface near base; III, IV, - lateral thoracic; V, VI, - lateral abdominal. The four dilutions of each of the three oils were assigned to the groups and sites as shown in Part IV, Appendix I, p.i. Some results are given in Table XVI. They have been further analysed statistically by Irwin (Part IV). Sections of skin from the mice which received more than 12 weeks' application, and the treated patches from the rabbits were cut and stained for examination of histological changes including classification of tumours.

Tests on 'experimentally derived' fractions (Birmingham only)

Some tests on materials derived from oil fractions during Professor Cook's and Dr. Carruther's researches were also made by application to the skin of mice and by subcutaneous injection.

1. An homogeneous crystalline solid (shown to be a homologue of chrysene) which was isolated from the extract Edmor 33 and designated Edmor 33A. This was injected subcutaneously into 24 mice; one sarcoma occurred after 24 months.

2. A viscous oil which was obtained by decomposition of a crystalline picrate prepared from an extract of a Lagunillas (Venezuela) crude. This was applied to the skin of 40 mice, 1% in benzene, and produced papillomata in 3 mice after 52, 56, and 60 weeks, respectively.

Period 1950-1951 - Tests on 3 selected crudes and 15 derived fractions

These tests were carried out in duplicate, at Birmingham and the Chester Beatty Research Institute. The source, quantities required

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12. The letters and numbers in brackets are the short form of reference to each dilution.

Table XIIa. Birmingham Dilution Tests. Mice Life Sheet, Solution D/1

Weeks from Start of Exparimont	Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
13	36	0	0	0
14	36	0	0	0
15	36	2	5.5	0
16	36	3	8.3	0
17	36	3	8.3	0
18	36	6	16.7	0
19	35	8	22.8	0
20	35	8	22.8	0
21	34	8	23.5	0
22	34	8	23.5	0
23	34	13	38.2	0
24	33	16	48.5	1
25	33	17	[illegible].5	1
26	32	19	59.4	1
27	29	18	62.1	3
28	28	18	64.3	4
29	25	16	64.0	6
30	24	15	64.2	7
31	24	16	64.7	7
32	23	16	72.7	8
33	21	14	66.7	10
34	19	15	78.9	11
35	18	15	83.3	12
36	15	15	100.0	15
37	15	15	100.0	15
38	12	12	100.0	18
39	11	11	100.0	19
40	11	11	100.0	19
41	10	10	100.0	20
42	9	9	100.0	21
43	3	3	100.0	27
44	2	2	100.0	28
45	2	2	100.0	28
46	1	1	100.0	29
47	1	1	100.0	29
48	1	1	100.0	29
49	1	1	100.0	29
50	1	1	100.0	29
51	0	0	-	30
52	0	0	-	30

Table XIIb. Birmingham Dilution Tests. Mice Life Sheet. Solution D/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	29	0	0	0
21	29	0	0	0
22	29	0	0	0
23	29	1	3.4	0
24	29	1	3.4	0
25	29	1	[illegible]	0
26	28	1	3.6	0
27	26	1	3.8	0
28	26	1	3.8	0
29	26	1	3.8	0
30	25	1	4.0	0
31	24	1	4.2	0
32	21	1	4.8	0
33	20	2	10.0	0
34	19	2	10.5	0
35	18	2	11.1	0
36	18	2	11.1	0
37	18	3	16.7	0
38	18	3	16.7	0
39	15	2	13.3	1
40	14	1	7.1	2
41	12	1	8.3	2
42	12	2	16.6	2
43	11	2	18.2	2
44	10	2	20.0	3
45	10	2	20.0	3
46	10	2	20.0	3
47	10	2	20.0	3
48	10	2	20.0	3
49	10	2	20.0	3
50	8	1	12.5	4
51	8	1	12.5	4
52	6	1	16.7	4
53	6	1	16.7	4
54	5	1	16.7	4
55	6	1	16.7	4
56	6	1	16.7	4
57	5	2	40.0	4
58	5	2	40.0	4
59	5	2	40.0	4
60	5	2	40.0	4
61	5	2	40.0	4
62	5	2	40.0	4
63	5	2	40.0	4
64	0	0	-	6

Table XIIc. Birmingham Dilution Tests. Mice Life Sheet. Solution D/16

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
45	15	0	0	0
46	12	0	0	0
47	11	0	0	0
48	11	0	0	0
49	11	0	0	0
50	11	0	0	0
51	11	2	18.2	0
52	10	2	20.0	0
53	10	2	20.0	0
54	8	1	12.5	1
55	8	1	12.5	1
56	6	0	0	2
57	6	0	0	2
58	6	0	0	2
59	6	0	0	2
60	4	0	0	2
41	4	0	0	2
62	4	0	0	2
63	4	0	0	2
64	0	0	-	2

Table XIId. Birmingham Dilution Tests. Mice Life Sheet. Solution D/64

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
45	16	0	0	0
46	14	0	0	0
47	12	0	0	0
48	11	0	0	0
49	11	0	0	0
50	11	0	0	0
51	11	1	16.1	0
52	9	1	11.1	0
53	9	1	11.1	0
54	9	1	11.1	0
55	8	1	12.5	0
56	8	1	12.5	0
57	8	1	12.5	0
58	8	1	12.5	0
59	7	1	14.3	0
60	7	1	14.3	0
61	7	1	14.3	0
62	7	1	14.3	0
63	5	0	0	1
64	0	0	-	1

Table XIIe. Birmingham Dilution Tests. Mice Life Sheet. Fraction I/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
15	34	0	0	0
16	33	0	0	0
17	33	0	0	0
18	33	1	3.0	0
19	33	1	3.0	0
20	33	1	3.0	0
21	33	1	3.0	0
22	32	1	3.1	0
23	32	3	9.4	0
24	32	5	15.6	0
25	31	6	19.3	0
26	31	7	23.5	0
27	31	7	23.5	0
28	30	10	33.3	1
29	29	11	37.9	2
30	28	13	46.4	2
31	27	15	55.5	3
32	27	15	55.5	3
33	27	16	59.2	3
34	26	16	61.5	4
35	26	16	61.5	4
36	24	14	58.3	6
37	24	17	70.8	6
38	24	17	70.8	6
39	24	18	75.0	6
40	19	16	84.2	11
41	17	14	82.3	13
42	17	14	82.3	13
43	13	10	76.9	17
44	12	10	83.3	18
45	10	9	90.0	20
46	6	6	100.0	24
47	5	5	100.0	25
48	5	5	100.0	25
49	5	5	100.0	25
50	5	5	100.0	25
51	4	4	100.0	26
52	3	3	100.0	27
53	3	3	100.0	27
54	2	2	100.0	28
55	2	2	100.0	28
56	2	2	100.0	28
57	2	2	100.0	28
58	2	2	100.0	28
59	2	2	100.0	28
60	2	2	100.0	28
61	2	2	100.0	28
62	2	2	100.0	28
63	2	2	100.0	28
64	2	2	100.0	28
65	2	2	100.0	28
66	1	1	100.0	29
67	0	0	-	30

Table XIIf. Birmingham Dilution Tests. Mice Life Sheet. Fraction I/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
40	14	0	0	0
41	12	1	8.3	0
42	10	1	10.0	0
43	9	0	0	1
44	8	0	0	1
45	8	0	0	1
46	8	0	0	1
47	8	0	0	1
48	8	0	0	1
49	6	0	0	1
50	5	0	0	1
51	5	0	0	1
52	5	0	0	1
53	5	0	0	1
54	5	0	0	1
55	5	0	0	1
56	5	0	0	1
57	5	0	0	1
58	4	0	0	1
59	4	0	0	1
60	4	0	0	1
61	4	0	0	1
62	4	0	0	1
63	4	0	0	1
64	4	0	0	1
65	3	0	0	1
66	3	0	0	1
67	0	0	-	1

Table XIIg. Birmingham Dilution Tests. Mice Life Sheet. Fraction F/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	32	0	0	0
21	32	0	0	0
22	32	0	0	0
23	31	2	6.4	0
24	31	2	6.4	0
25	31	3	9.6	0
26	27	3	11.1	1
27	22	3	13.6	2
28	22	4	18.2	3
29	18	3	16.7	3
30	17	5	29.4	4
31	14	5	35.7	4
32	14	5	35.7	4
33	11	6	54.5	5
34	11	6	54.5	5
35	11	7	63.6	5
36	9	7	77.8	5
37	9	7	77.8	5
38	9	7	77.8	5
39	9	7	77.8	5
40	7	5	71.4	7
41	6	4	66.7	8
42	6	3	50.0	9
43	5	3	60.0	9
44	5	4	80.0	9
45	4	3	75.0	10
46	4	3	75.0	10
47	3	3	100.0	11
48	2	2	100.0	12
49	1	1	100.0	13
50	1	1	100.0	13
52	0	0	-	14

Table XIIIa. London Dilution Tests. Mice Life Sheet. Fraction D/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
12	29	0	0	0
13	29	4	13.8	0
14	29	5	17.2	0
15	29	5	17.2	0
16	29	5	17.2	0
17	29	6	20.7	0
18	29	7	24.1	0
19	27	10	37.0	2
20	26	10	38.5	2
21	24	10	41.7	4
22	24	10	41.7	4
23	24	10	41.7	4
24	21	15	71.4	7
25	19	14	73.7	8
26	16	11	68.8	11
27	16	11	68.8	11
28	12	11	91.7	15
29	12	11	91.7	15
30	11	10	90.9	16
31	11	10	90.9	16
32	10	9	90.0	17
33	9	8	88.9	18
34	5	5	100.0	22
35	5	5	100.0	22
36	4	4	100.0	23
37	3	3	100.0	24
38	3	3	100.0	24
39	2	2	100.0	25
40	2	2	100.0	25
41	2	2	100.0	25
42	2	2	100.0	25
43	2	2	100.0	25
44	2	2	100.0	25
45	2	2	100.0	25
46	0	0	0.0	27

Table XIIIb. London Dilution Tests. Mice Life Sheet. Fraction D/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
18	41	0	0	0
19	39	1	2.6	0
20	39	1	2.6	0
21	38	4	10.5	1
22	38	4	10.5	1
23	38	4	10.5	1
24	38	4	10.5	1
25	38	5	13.2	1
26	38	5	13.2	1
27	38	5	13.2	1
28	37	8	21.6	2
29	35	10	28.6	3
30	35	12	34.3	3
31	35	12	34.3	3
32	33	10	30.3	5
33	33	11	33.3	5
34	32	16	50.0	5
35	29	13	44.8	8
36	29	13	44.8	8
37	29	13	44.8	8
38	29	13	44.8	8
39	27	17	63.0	10
40	27	17	63.0	10
41	27	22	81.5	10
42	25	20	80.0	12
43	24	19	79.2	13
44	19	14	73.7	18
45	19	14	73.7	18
46	19	14	73.7	18
47	15	12	80.0	20
48	15	12	80.0	20
49	15	14	93.3	20
50	13	12	92.3	22
51	12	12	100.0	22
52	11	11	100.0	23

Table XIIIc. London Dilution Tests. Mice Life Sheet. Fraction D/16

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	39	0	0	0
21	39	2	5.1	0
22	39	2	5.1	0
23	39	2	5.1	0
24	39	2	5.1	0
25	39	2	5.1	0
26	38	1	2.6	1
27	38	1	2.6	1
28	37	1	2.7	1
29	37	1	2.7	1
30	35	0	0.0	2
31	35	0	0.0	2
32	35	0	0.0	2
33	34	0	0.0	2
34	34	0	0.0	2
35	34	0	0.0	2
36	33	2	6.1	2
37	33	2	6.1	2
38	33	2	6.1	2
39	33	2	6.1	2
40	33	2	6.1	2
41	33	2	6.1	2
42	33	2	6.1	2
43	33	2	6.1	2
44	33	2	6.1	2
45	33	2	6.1	2
46	33	2	6.1	2
47	31	2	6.5	2
48	31	2	6.5	2
49	31	3	9.7	2
50	31	3	9.7	2
51	31	3	9.7	2
52	30	8	26.7	2

Table XIIId. London Dilution Tests. Mice Life Sheet. Fraction I/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
12	44	0	0	0
13	43	1	2.3	0
14	42	1	2.4	0
15	41	1	2.4	0
16	41	1	2.4	0
17	41	1	2.4	0
18	41	1	2.4	0
19	41	1	2.4	0
20	41	1	2.4	0
21	41	1	2.4	0
22	41	1	2.4	0
23	41	1	2.4	0
24	39	1	2.6	0
25	39	2	5.1	0
26	37	2	5.4	0
27	37	2	5.4	0
28	34	2	5.9	0
29	34	2	5.9	0
30	34	2	5.9	0
31	34	2	5.9	0
32	34	2	5.9	0
33	34	2	5.9	0
34	34	2	5.9	0
35	33	2	6.1	0
36	33	2	6.1	0
37	33	2	6.1	0
38	31	1	3.2	1
39	31	2	6.5	1
40	31	2	6.5	1
41	31	2	6.5	1
42	31	2	6.5	1
43	30	2	6.7	1
44	30	2	6.7	1
45	30	2	6.7	1
46	30	5	16.7	1
47	30	5	16.7	1
48	30	5	16.7	1
49	30	9	30.0	1
50	30	9	30.0	1
51	30	9	30.0	1
52	30	10	33.3	1

Table XIIIe. London Dilution Tests. Mice Life Sheet. Fraction F/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
15	45	0	0	0
16	45	1	2.2	0
17	45	1	2.2	0
18	45	1	2.2	0
19	45	1	2.2	0
20	44	1	2.3	0
21	44	1	2.3	0
22	43	1	2.3	0
23	42	1	2.4	0
24	42	1	2.4	0
25	42	1	2.4	0
26	42	1	2.4	0
27	41	1	2.4	0
28	40	1	2.5	0
29	39	1	2.6	0
30	37	1	2.7	0
31	37	2	5.4	0
32	37	2	5.4	0
33	37	2	5.4	0
34	37	2	5.4	0
35	37	2	5.4	0
36	37	3	8.1	0
37	37	3	8.1	0
38	34	2	5.9	1
39	34	2	5.9	1
40	34	2	5.9	1
41	33	2	6.1	1
42	33	2	6.1	1
43	33	2	6.1	1
44	33	2	6.1	1
45	33	2	6.1	1
46	33	2	6.1	1
47	33	2	6.1	1
48	33	2	6.1	1
49	33	2	6.1	1
50	32	2	6.3	1
51	31	2	6.5	1
52	30	2	6.7	1

Table XIV. Dilution Tests. Survival Rate of Mice

Material	Number of Mice out of 50 surviving					
	12 weeks		25 weeks		50 weeks	
	B	L	B	L	B	L
D/1	36	29	33	19	0	0
D/4	34	41	29	38	8	13
D/16	34	41	30	39	11	31
D/64	48	44	42	44	11	36
I/1	36	44	31	39	5	30
I/4	36	41	33	38	5	32
I/16	34	49	25	48	6	37
I/64	34	44	30	43	8	33
F/1	35	45	31	42	5	32
F/4	44	47	44	47	17	36
F/16	40	43	36	40	19	29
F/64	26	46	17	43	9	35

B = Birmingham L = London

Table XV. Dilution Tests. Tumour Incidence in Mice

Material	Week in which 1st tumour appeared		No. of tumours elicited at 25 weeks		Total No. of tumours out of 50 possible	
	B[x]	L[ϕ]	B	L	B	L
D/1	15th	14th	20	22	30	27
D/4	23rd	20th	1	5	5	34
D/16	51st	22nd	0	2	2	10
D/64	51st	-	0	0	1	0
I/1	18th	14th	7	1	30	11
I/4	41st	-	0	0	1	0
I/16	?1 in 41st week, re-gressed, not visible at 44 weeks	-	0	0	0	0
I/64	0	-	0	0	0	0
F/1	23rd	17th	4	1	14	3
F/4	0	-	0	0	0	0
F/16	0	-	0	0	0	0
F/64	0	-	0	0	0	0

[x] B = Birmingham [ϕ] L = London

Table XVI. Birmingham Dilution Tests. Tumour Incidence in Rabbits

Material	Week in which first tumour appeared	Sites with tumours at 52 weeks			Time for 4 tumours (22%)	Time for 50% incidence of tumours	
		Total	Body	Ear		Total	Body
D/1	34th	11/18	7/12	4/6	40 wks	49 wks	42 wks
D/4	34th	4	3	1	48 wks	-	-
D/16	-	0	0	0	-	-	-
D/64	-	0	0	0	-	-	-
I/1	33rd	9/18	5/12	4/6	40 wks	-	-
I/4	34th	8	4	4	42 wks	-	-
I/16	48th	2	1	1	-	-	-
I/64	-	0	0	0	-	-	-
F/1	36th	11/18	8/12	3/6	41 wks	45 wks	45 wks
F/4	46th	1	1	0	-	-	-
F/16	47th	1	0	1	-	-	-
F/64	-	0	0	0	-	-	-

Total number of rabbit sites = 216, 144 on the body and 72 on the ears.

There are 18 rabbit sites to each fraction, 12 on body and 6 on ears.

Table XVII. Carcinogenic activity of fractions of oils

Number of tumours at the Birmingham Laboratory = B
Number of tumours at the London Laboratory = L

Oil	(Fractions arranged in order light ⟶ heavy)		Maximum possible No. of tumours	Rabbit test Number of tumours B/L	Mouse Test Number of tumours B/L	Maximum possible No. of tumours
Kuwait	crude oil	K	60	0/0	2/0	50
	light fraction	K1	35	1/0	2/0	"
	↓	K2	"	3/1	4/1	"
		K3	"	5/4	3/0	"
	heavy fraction	K4	"	12/8	2/1	"
	residue	K5	"	0/0	1/0	"
Laguinillas	crude oil	L	60	0/0	0/0	"
	light fraction	L1	35	1/0	2/0	"
	↓	L2	"	4/2	5/0	"
		L3	"	13/3	5/2	"
	heavy fraction	L4	"	16/6	3/1	"
	residue	L5	"	0/2	0/0	"
Oklahoma	crude oil	0	60	0/2	0/0	"
	light fraction	01	35	0/1	0/0	"
	↓	02	"	1/2	0/0	"
		03	"	1/4	2/1	"
	heavy fraction	04	"	4/4	2/0	"
	residue	05	"	0/0	0/0	"

1952 - Work in progress

After comparing the results of the Dilution Experiments in which the 9:10-dimethyl-1:2-benzanthracene was used at 0.1% - 0.0015% in liquid paraffin, with those obtained in the experiments on the derived fractio of O, K, and L, Professor Squire suggested that some idea of the content of carcinogen of the various fractions in terms of 'equivalent' of that polycyclic hydrocarbon could be computed. Similarly, the equivalent concentration in the original crude could be estimated and thus an idea obtained of the degree of concentration achieved in the derived fractions. Thus K_4 and L_4 appear to have about 7 and 9 times the dimethylbenzanthracene 'equivalent' of the original crude.

Since it was possible that further considerable 'concentration' of carcinogen might be expected to be attained by separating those active fractions into cuts of narrower temperature limits, a scheme was devised for testing 5 sub-fractions from each and incorporating two dilutions of three possible reference standards.

The 15 sub-fractions separated under the supervision of Professor Morton are shown in Table I. The reference standards comprise:-

9:10-dimethyl-1:2-benzanthracene, 0.2% and 0.05% in liquid paraffin;

Methylcholanthrene 0.2% and 0.5% in liquid paraffin;

Kuwait fraction 4, neat and diluted 4 times with liquid paraffin.

These 21 specimens are now being tested on a total of 1050 mice and 105 rabbits.

The allocation of sites for the rabbit applications, devised by Dr. Irwin and Dr. Armitage is given in Table XIX, together with the appropriate key. As will be noted, 5 rabbit sites are involved, the 2 ears, left and right thoracic, and interscapular. The experimental procedure previously used is being followed.

Table XIX. Design of experiment on 21 fractions. (1952 tests)

Rabbits	I	II	III	IV	V
1 - 5	d	p	n	u	s
6 - 10	e	q	o	s	b
11 - 15	f	r	p	b	c
16 - 20	g	s	q	c	d
21 - 25	h	t	r	d	e
26 - 30	i	u	s	e	f
31 - 35	j	a	t	f	g
36 - 40	k	b	u	g	h
41 - 45	l	c	a	h	i
46 - 50	m	d	b	i	j
51 - 55	n	e	c	j	k
56 - 60	o	f	d	k	l
61 - 65	p	g	e	l	m
66 - 70	q	h	f	m	n
71 - 75	r	i	g	n	o
76 - 80	s	j	h	o	p
81 - 85	t	k	i	p	q
86 - 90	u	l	j	q	r
91 - 96	a	m	k	r	s
97 - 100	b	n	l	s	t
101 - 105	c	o	m	t	u

Key to fractions a - u

$K_{4.1} = k$
$K_{4.2} = b$
$K_{4.3} = u$
$K_{4.4} = m$
$K_{4.5} = a$

$O_{4.1} = c$
$O_{4.2} = t$
$O_{4.3} = r$
$O_{4.4} = j$
$O_{4.5} = e$

$L_{4.1} = f$
$L_{4.2} = s$
$L_{4.3} = a$
$L_{4.4} = l$
$L_{4.5} = d$

$M_2 = h$
$M_{\frac{1}{2}} = p$
$D_2 = c$
$D_{\frac{1}{2}} = q$
$K_4 = n$
$K_{4.\frac{1}{4}} = i$

$a = L_{4.3}$
$b = K_{4.2}$
$c = D_2$
$d = L_{4.5}$
$e = O_{4.5}$
$f = L_{4.1}$

$g = K_{4.5}$
$h = M_2$
$i = K_{4.\frac{1}{4}}$
$j = O_{4.4}$
$k = K_{4.1}$
$l = L_{4.4}$

$m = K_{4.4}$
$n = K_4$
$o = O_{4.1}$
$p = M_{\frac{1}{2}}$
$q = D_{\frac{1}{2}}$
$r = O_{4.3}$

$s = L_{4.2}$
$t = O_{4.2}$
$u = K_{4.3}$

Table XX. Experiments with mice at Birmingham. Three crudes and fifteen derived fractions

Crude oil or fraction	Expectation of tumourless life and standard error (limited to 52 weeks)	Dates when 10% of survivors had tumours and standard error	Total number of tumours
Crude Kuwait	50.34 ± 1.16	39.4 ± 9.3	2
Kuwait 1	50.73 ± 0.89	39.5 ± 6.4	2
" 2	47.42 ± 2.11	28.9 ± 2.8	4
" 3	48.10 ± 2.13	27.8 ± 1.0	3
" 4	50.19 ± 1.26	not reached	2
" Residue	51.06 ± 0.94	not reached	1
Crude Laguinillas	52	no tumours	0
Laguinillas 1	50.48 ± 1.10	not reached	2
" 2	48.67 ± 1.48	28.7 ± 0.8	5
" 3	48.75 ± 1.85	30.4 ± 1.5	5
" 4	48.98 ± 1.66	31.8 ± 1.2	3
" Residue	52	no tumours	0
Crude Oklahoma	52	no tumours	0
Oklahoma 1	52	no tumours	0
" 2	52	no tumours	0
" 3	50.04 ± 1.35	32.5 ± 3.1	2
" 4	49.92 ± 1.43	46.5 ± 4.3	2
Residue	52	no tumours	0

Table XVIII. Carcinogenic activity of fractions of oils in mouse tests

Date of appearance of each tumour (weeks from start) and numbers of survivors at that time

Fraction		Birmingham	London	
	No. of tumours	Weeks from start and no. of survivors*	No. of tumours	Weeks from start & no. of survivors
K crude	2	29/27, 32/26,	0	-
K1	2	30/25, 32/29,	0	-
K2	4	21/24, 28/20, 31/18,	1	34/25
K3	3	23/23, 27/18, 28/18,	0	-
K4	2	30/24, 32/23,	1	52/36
K5 (residue)	1	30/24	0	-
L crude	0	-	0	-
L1	2	26/27, 41/20,	0	-
L2	5	28/29, 28/29, 29/27, 44/16, 48/14,	0	-
L3	5	28/24, 28/24, 31/24, 32/23, 48/13	2	26/34, 52/19
L4	3	30/21, 32/18,	1	52/32
L5 (residue)	0	-	0	-
O crude	0	-	0	-
O1	0	-	0	-
O2	0	-	0	- -
O3	2	30/23, 30/23,	1	31/40
O4	2	25/21, 38/13,	0	-
O5 (residue)	0	-	0	-

* The first figure is the date of appearance (e.g. 29 means the 29th week of the experiment), the second is the number of survivors out of 50.

and the scope of the initial fractionation of the crudes to be examined were considered in committee from all aspects (see also Part IV, Appendix I).

The crudes were (1) an asphaltic, paraffinic type (Kuwait), (2) a naphthenic type (Lagunillas (Venezuela)), and (3) a paraffinic type (Oklahoma, U.S.A. Mid Continental). Each of these was separated into 5 fractions at the Thornton Research Centre.

The tests for carcinogenic activity were carried out as before on groups of 50 mice and on a total of 105 rabbits. For the 3 crudes a total of 30 rabbits were used with 6 skin patches on each and for the 15 derived fractions a total of 75 rabbits with 7 application sites. These were the same as those described in the section on 'Dilution Experiments' with the addition of one 'interscapular' site.

The allocation of the animals and sites is set out in Part IV, Appendix I, p. i, where for Birmingham, A = Oklahoma, B = Lagunillas, C = Kuwait, and for London A = Kuwait, B = Lagunillas and C = Oklahoma. (see also Table XIX).

Results

Mouse tests

The numbers of tumours induced by the crudes and fractions are given in Table XVII and the time of appearance of all the tumours is given in Table XVIII. The total numbers of tumours obtained were 33 and 6 respectively (in 900 mice) for Birmingham and London, and the greatest number for any one fraction was 5 (in 50 mice). The results suggest that the Lagunillas derived fractions were somewhat more active than the Kuwait and that the Oklahoma fractions were essentially inert.

At Birmingham only two tumours were obtained after continued application of the crudes; at London they yielded no tumours after 50 weeks.

Rabbit tests

In contrast with the tests on mice, there was a total of 61 tumour-bearing sites in the Birmingham series and 39 in the London series, on a possible 705 sites (105 rabbits), or, excluding the crudes and residues (fraction 5), the remaining 12 fractions produced 61 and 35 tumours respectively on a possible 420 sites (60 rabbits) (Table XVII). Thus the skin of the rabbit was distinctly more sensitive to these fractions than that of the mouse. This observation was confirmed by the type and rate of growth of the papillomata. In a number of instances, at both laboratories, several tumours occurred on one rabbit site. In general the grading of the carcinogenic potency ran similarly in the Birmingham and London tests. Sections of skin from the treated areas have been prepared for histological examination.

Irwin's statistical analysis brings out the following points. Fractions L_1, L_5; O_1, O_2, O_5; K_1, K_2, K_5 can be regarded as of negligible carcinogenic potency. According to the Birmingham figures the order of potency for the fractions 4, the most potent, is L_4, K_4, O_4; and the London results are not inconsistent with this.

A striking feature of the tests is the special sensitivity of rabbit skin compared with mouse skin for these mineral oil fractions. The response of different species of animals to different carcinogens has been investigated by a number of workers including Boyland; some pure chemical carcinogens have selective activity towards certain species. It may be no more than a conjecture to suggest that the results indicate the presence of carcinogens differing in chemical structure from the better known polycyclic hydrocarbons. However, they reinforce the opinion that the rabbit should be included in tests for 'carcinogenicity' on this type of material.

Table XXI. Experiments on rabbits with 15 derived fractions. Mean values (corrected for group differences)

Fraction	Birmingham		Chester Beatty Institute		Total no. of tumours	
	Transformed Value (y)	Probability	Transformed Value (y)	Probability	Birmingham	Chester Beatty
L_1	4.27	0.007*	(-3.12)	0.000	1	0
L_2	14.89	0.066	5.52	0.010	4	2
L_3	34.58	0.323	13.52	0.055	13	3
L_4	43.51	0.473	19.38	0.10:	16	6
L_5	-1.35	0.000	8.15	0.020	0	2
K_1	3.97	0.007	0.25	0.000	1.	0
K_2	8.52	0.022	2.90	0.005	3	1
K_3	17.80	0.093	13.44	0.054	5	4
K_4	30.95	0.264	24.2:	0.168	12	8
K_5	-0.02	0.000	2.23	0.004	0	0
O_1	1.25	0.002	3.70	0.006	0	1
O_2	2.64	0.005	5.59	0.010	1	2
O_3	5.24	0.009	14.62	0.064	1	4
O_4	8.99	0.025	13.80	0.057	4	4
O_5	1.27	0.002	0.25	0.000	0	0
SE individual means	5.15		4.16			
SE diff. bet. 2 means	7.29		5.88			

* The unbracketed fractions are of negligible carcinogenicity.

Section I

STATISTICS AND METHODS

The plan of the experiments and the instructions for carrying them out are shown in Appendix I. The experiments were of two kinds:-

1. Experiments on the three whole crudes and five fractions derived from each of them.

2. Dilution experiments.

Both sets were carried out on rabbits as well as mice. The three crudes were Kuwait, Lagunillas and Oklahoma. The methods of distillation and the physical characteristics of the fractions are described in Appendix II. Fraction 5 was in each case the residue.

1. Experiments on the three whole crudes and fifteen derived fractions

In the mouse experiments a batch of 50 mice was used for each crude and fraction both at Birmingham and at the Chester Beatty Research Institute. At the Chester Beatty Institute there were only 6 tumours in 900 mice; these results were clearly not worth while analysing statistically. At Birmingham there were very few tumours and no significant differences between any of the oils (crudes or fractions). Table XX shows for each of these fractions the expectation of tumourless life (limited to 52 weeks), the dates when 10 per cent of surviving mice had tumours together with their standard errors. The total number of tumours is also given. The distribution of the number of tumours was as follows:-

No. of tumours	No. of fractions	
	Observed	Expected (Poisson)
0	6	3
1	1	5
2	6	5
3 or more	5	5
	18	18
$\chi^2 = 6.2$		2 d.f.

There is no significant departure from the frequencies expected on the basis of chance variation about a fixed mean (Poisson frequencies).

The other two measures of response also show no significant differences.

For the rabbit experiments on the three whole crudes a 6 x 6 Latin Square design was used. (see Appendix I). For the experiments on the fifteen derived fractions a more complicated design (known as a Youden square) was employed. Here there were 15 groups of five rabbits. In each group each rabbit was painted with the same 7 fractions. Each of the 15 fractions was painted on each site in one and only one group. Each fraction was used in exactly 7 groups and every pair of fractions was used in exactly 3 groups. To obtain the designs actually used at

Birmingham and the Chester Beatty Research Institute we must substitute in the key of Appendix I:- for Birmingham (A = Oklahoma, B = Legunillas, C = Kuwait) and for the Chester Beatty Research Institute (A = Kuwait, B = Legunillas, C = Oklahoma).

In the experiments with the three whole crudes no tumours were obtained at Birmingham and only 2 tumours on 180 sites at the Chester Beatty Research Institute; thus, there was no need for statistical analysis in these two experiments. Results of great interest were, however, obtained from the experiments with the fifteen derived fractions.

The percentage of each group of five animals which got tumours on each site was calculated. The method of analysis employed was to transfer percentages to degrees by means of the angular transformation $p = \sin^2 y$ (which approximately equalises the variance at different levels) and then to perform an analysis of variance of the results. Table XXI shows the mean values of the transformed variable, and the probabilities of getting a tumour, estimated for each fraction. Fractions L_1, L_5, K_1, K_2, K_5, O_1, O_2, O_5 can be regarded as of negligible potency; so can O_3, O_4 in the Birmingham results but not in the Chester Beatty Institute's results, and L_2 in the Chester Beatty Institute's but not in the Birmingham results. If we exclude the eight fractions first mentioned we are left with seven; L_2, L_3, L_4, K_3, K_4, O_3, O_4. The Birmingham results show that fraction 4 is more potent than fraction 3 and that the order of potency is $L > K > O$. Further, these differences in potency are significant. The results from the Chester Beatty Institute for these fractions show no significant differences among themselves (except for L_2) but are not incompatible with the Birmingham results.

The analysis of variance shows that there are significant differences in sensitivity between the fifteen groups of rabbits in both sets of results. In the Birmingham experiment, site differences might be due to chance ($P = 0.12$ for greater variance ratio than that observed), in that at the Chester Beatty Institute they just pass the 5 per cent level of significance ($P = 0.04$). However, the actual results shown in Table XXIII are concordant and suggest a real effect. Both series show the greatest number of tumours on site VII (in the middle of the back nearest the ears) and sites I and II (the ears) come next on the average. There seems to be a larger number of tumours nearer the head.

2. Dilution experiments

As stated in Section II of Appendix I the pure carcinogen was 9:10-dimethyl-1:2-benzanthracene. The other two oils were the fractions I and F which had been tested before at Birmingham during 1944-6 and 1946-7. (Woodhouse and Irwin 1950). In the mouse experiments batches of 50 mice were used for each oil in each centre (Birmingham and the Chester Beatty Institute). The results are given in Table XXIV. Graded dosage response curves were obtained for all three oils at Birmingham, and for the pure carcinogen in both centres. It clearly seems best to use E.T.L. as a response in these experiments since the 10 per cent dates are not always reached. Between the undiluted oil and the 1/4 dilution the slopes of these 4 dosage-response curves do not differ significantly. At Birmingham the same is true of the slopes between the 1/4 and 1/16 dilution, for all three oils. This result is very encouraging as it suggests that dimethyl-benzanthracene might be used as a standard in comparing the carcinogenic potencies of the oil fractions.

Owing to changes of staff, the rabbit dilution experiments were completed only at Birmingham.

The design for the rabbit experiments (see Appendix I (2)) consisted essentially of three 4 x 4 Latin Squares, using sites III, IV, V and VI. Sites I and II, being available, were used for some additional observations, so as to permit if necessary of direct comparisons between different fractions at the same dilution in the same rabbits. However, in the event, the statistical analysis of the three 4 x 4 Latin Squares, was sufficient to show that there were no significant differences between the dosage-response curves of the three fractions. This analysis is presented here.

Table XXII. Experiment with rabbits on fifteen derived fractions

Analysis of variance on angular transformation of proportion of tumours out of 5

Birmingham

	Sum of squares	Degrees of freedom	Mean Square	Variance Ratio
Sites	1957.36	6	326.23	1.84 (not sign.)
Groups of rabbits	5772.04	14	412.29	2.33 (0.01 < P < 0.05)
Fractions	18924.47	14	1351.78	7.64 (P < 0.01)
Error	12384.47	70	176.92	
	39038.81	104		

Chester Beatty Institute

	Sum of squares	Degrees of freedom	Mean Square	Variance Ratio
Sites	1613.75	6	268.96	2.36 (0.01 ≤ P < 0.05)
Groups of rabbits	4980.42	14	355.74	3.12 (P < 0.01)
Fractions	5561.15	14	397.23	3.48 (P < 0.01)
Error	7983.01	70	114.04	

(The 'theoretical' error variance is 820.7/5 = 164.1, with which the above estimates are in good agreement.)

Table XXIII. Experiment with rabbits on fifteen derived fractions

Number of tumours out of 75 on the seven different sites

Site	Birmingham	Chester Beatty Institute	Mean
I	11	6	8.5
II	11	7	9.0
III	12	2	7.0
IV	4	4	4.0
V	3	4	3.5
VI	7	3	5.0
VII	13	11	12.0

analysed. For any one dose and site there are 2 degrees of freedom for difference between the three rabbits making 32 in all (4 sites, 4 groups of rabbits, 4 x 4 x 2 = 32). These 32 together with the 15 from the Latin Square analysis make 47 in all, clearly correct for 48 observations. The 32 degrees of freedom can be sub-divided into 8 due to differences between rabbits and 24 due to error. The analysis has been carried out by 90° scoring for a tumour and 0° for the absence of a tumour; the results are shown in Table XXVI.

The differences between rabbits are significant only for fraction I (Variance Ratio = 2.59, 5% pt = 2.36). There are no significant differences between sites. There are no significant differences between the three fractions at the same dilution, even if the smaller standard error, based on the assumption of homogeneity of the rabbits be used. Hence, it was unnecessary to use the supplementary observations mentioned above.

Table XXVI. Rabbit Dilution Experiments at Birmingham

Analysis of Variance of differences at the same sites and dilutions
(Tumour = 90° Absence of tumour = 0°
(Sums of Squares and mean squares divided by 3, to make them comparable with Table XXV.)

	Fraction D			
	Sum of Squares	Degrees of freedom	Mean Squares	Variance Ratio
Between rabbits	2700	8	337.5	1.29
Error	6300	24	262.5	
Total	9000	32		

	Fraction I			
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Between rabbits	5625	8	703.12	2.42 sign. at 5%
Error	6975	24	290.62	
Total	12600	32		

	Fraction F			
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Between rabbits	2250	8	281.25	1.36
Error	4950	24	206.25	
Total	7200	32		

Rather unexpectedly there were no significant differences between rabbits treated alike (except possibly for fraction I) in the probability of getting a tumour; nor were there significant differences between sites treated alike.

The method of analysis employed was to transfer percentages to degrees by means of the angular transformation $p = \sin^2 y$ (which approximately equalises the variance at different levels) and then to perform an analysis of variance of the results. The regression of y on log-dilution was calculated and did not differ significantly from linearity. The error variances obtained from the experimental results were in all cases in good agreement with their theoretical values.

$$\left\{ \frac{1}{12} \left(\frac{180}{} \right)^2 = 273.6 \right\}$$

This justifies the method of analysis.

From the regressions an estimate of the probability of getting a tumour at the four dilution levels was estimated for each fraction. The results are:-

Dilution	Fraction			All Three	5% Fiducial limits
	D Prob.	I Prob.	F Prob.		
1	0.53	0.33	0.49	0.45	0.30-0.75
1/4	0.22	0.16	0.19	0.19	0.11-0.36
1/16	0.03	0.04	0.02	0.03	0.00-0.10
1/64	0.00	0.00	0.00	0.09	0.00-0.01

These results are not significantly different for the different oils, so that the estimates in the last two columns can be taken as applying to each of them.

The error variances per group of 3 rabbits taken from the analyses of variance are

Fraction	Error Variance (6 d.f.)
D	218.4
I	213.1
F	288.3
Theoretical value	273.6

The detailed analyses of variance are shown in Tables XXV and XXVI.

The Latin Squares are analysed in the usual manner; the 3 degrees of freedom for differences between the four dilutions have been sub-divided into 1 due to the linear regression of response (y) on the logarithm of the dilution and the remaining 2 due to departure from linear regression. This departure is not significant. These results are given in Table XXV.

To investigate whether there are any significant differences between rabbits treated alike, the results for individual rabbits must be

Table XXIV. Mouse Dilution Experiments

Expectation of tumourless life and dates when 10 per cent of survivors have tumours

	Birmingham			Chester Beatty Institute		
	E.T.L. (limited to 52 wks.)	10% Date	No. of tumours	E.T.L. (limited to 52 wks.)	10% Date	No. of tumours
D/1	28.17 ± 2.04	17.2 ± 0.6	30	22.06 ± 1.58	12.7 ± 0.3	27
D/4	48.08 ± 1.71	33.0	5	34.41 ± 1.45	21.0 ± 0.7	34
D/16	51.73 ± 0.23	50.5 ± 0.4	0	49.26 ± 1.27	not reached	10
D/64	51.86 ± 0.17	50.6 ± 0.4	0	52	"	0
I/1	31.91 ± 1.37	23.1 ± 0.8	30	48.84 ± 1.22	45.6 ± 1.0	11
I/4	51.18 ± 0.83	42.0	1	52	not reached	0
I/16	52	not reached	0	52	"	0
I/64	52	"	0	52	"	0
F/1	36.39 ± 2.57	25.3 ± 3.1	14	50.16 ± 1.08	not reached	3
F/4	52	not reached	0	52	"	0
F/16	52	"	0	52	not reached	0
F364	52	"	0	52	"	0

Table XXV. Rabbit Dilution Experiments at Birmingham. Analysis of Variance of Latin Squares

(Angular transformation of the proportion of tumours out of 3)

Fraction D

	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	1184.06	3	394.08	1.80
Sites	1714.65	3	571.55	2.62
Dilutions Regression	6769.36	1	6769.36	31.00 sign. at 1%
Dilutions Remainder	1018.65	2	509.37	2.32
Error	1310.22	6	218.37	
	11996.94	15		

Fraction I

	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	1944.98	3	664.99	3.12
Sites	779.57	3	259.86	1.22
Dilutions Regression	2713.29	1	2713.29	12.73 sign. at 5%
Dilutions Remainder	339.60	2	169.80	<1
Error	1278.51	6	213.08	
	7105.95	15		

Fraction F

	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	553.30	3	184.43	<1
Sites	210.89	3	70.30	<1
Dilutions Regression	6833.90	1	6833.90	23.71 sign. at 1%
Dilutions Remainder	2692.40	2	1346.20	4.67
Error	1729.63	6	288.27	
	12020.12	15		

(3) Recording of warts

Records will be maintained of the following observations:-

1. The date when a wart first appears.

2. The approximate date when a tumour is regarded as malignant on clinical grounds.

3. The date of death or killing.

Histological examination of all painted sites will be carried out, provided that death occurs after 12 weeks or after the appearance of the first tumour should this appear earlier.

APPENDIX I

THE DESIGN OF EXPERIMENTS FOR TESTING THE CARCINOGENICITY OF OIL FRACTIONS

I. Tests on 18 fractions, three whole crudes and five fractions derived from each of them.

(1) Mouse tests

The experiments are to be done in duplicate - on batches of 50 mice at Birmingham and 50 at the Chester Beatty Research Institute. These experiments should be standardised as far as possible, in particular in the following respects:

(a) Five or six animals per box.

(b) Sexes in equal numbers but separated. Age as near to 2 months as possible but in all cases less than 4 months.

(c) The 25 males and 25 females on each fraction to be selected randomly from the stock existing at the time the experiment is started. This ratio may have to be altered at the Chester Beatty Research Institute to include a higher proportion of males.

(d) Each animal in each test to be numbered and the sex and colour, including spotting if any, to be recorded. This is in order to be able to divide the animals in each test into random groups.

(e) Diet. Thorley's Rat Cubes, plus supplement as given at the Chester Beatty Research Institute, viz. bread and cod liver oil on Mondays, oats on Wednesday, and a small amount of greens on Fridays. Free water supply.

(f) Twice weekly painting with glass rod from stoppered numbered bottles, dispensed under single control at the Chester Beatty Research Institute.

(2) Rabbit tests

The following experiments are to be done in duplicate at Birmingham and the Chester Beatty Research Institute. Male or female rabbits to be used, between 3 and 6 months old. Sex and colour to be recorded as for mice. Diet as for mice. Two experiments are to be performed (a) on the three whole crudes, (b) on the fifteen fractions derived from them. Each rabbit is to be painted on six sites in (a) and on seven in (b) as follows:-

Sites I, II Ears, outer surface near base.

III, IV Lateral thoracic

V, VI Lateral abdominal

VII Interscapular

(a) Experiment on the three whole crudes. 30 rabbits are to be used and divided randomly into 6 groups of 5 rabbits. The three crudes A, B, C to be assigned to the groups and sites in accordance with the following scheme:-

SITES

Rabbits	I	II	III	IV	V	VI
1 - 5	B	B	C	C	A	A
6 - 10	C	C	A	A	B	B
11 - 15	A	A	B	B	C	C
21 - 25	C	C	B	B	A	A
26 - 30	A	A	C	C	B	B

Dates of appearance of tumours and of course any deaths are to be recorded. The experiment is to be continued for 12 months.

(b) Experiment on fifteen derived fractions

75 rabbits to be used and divided randomly into 15 groups of 5. The 15 fractions a, b, c, d.....o to be assigned to the groups and sites in accordance with the following scheme:-

Rabbits	I	II	III	IV	V	VI	VII
1 - 5	f	g	a	e	c	b	d
6 - 10	k	j	b	i	h	a	c
11 - 15	c	n	m	l	a	c	b
16 - 20	h	i	d	n	c	e	a
21 - 25	a	e	l	k	m	d	j
26 - 30	i	a	f	m	l	g	h
31 - 35	c	k	g	a	j	f	n
36 - 40	d	m	h	c	b	j	f
41 - 45	n	f	i	b	d	k	l
46 - 50	l	b	j	h	g	n	e
51 - 55	b	c	k	g	e	i	m
56 - 60	m	h	n	d	k	c	g
61 - 65	g	d	c	j	i	l	c
66 - 70	e	l	c	c	f	h	k
71 - 75	j	c	e	f	n	m	i

Key to fractions A - O

Fraction in order of light to heavy	A	B	C
1	k	c	n
2	h	m	i
3	j	f	g
4	a	b	d
5	c	e	l

or a = A4, b = B4, c = A5, d = C4, e = B5, f = B3, g = C3, h = A2, i = C2, j = A3, k = A1, l = C5, m = B2, n = C1, o = B1

Date of appearance of tumours and, of course, any deaths to be recorded. The experiment to be continued for 12 months

Tests on 4 dilutions of each of three 'oils', one of which is to be a pure carcinogen, 9:10-dimethyl-1:2-benzanthracene. The pure carcinogen is to be given at dilutions

1 = (0.1)% in medicinal liquid paraffin

1/4 = 0.025% " " "

1/16 = 0.00625% " " "

1/64 = 0.0015% " " "

The other two oils to be given at dilutions:-

1 = neat

1/4 = 25% in liquid paraffin

1/16 = 6.25% " "

1/64 = 1.5625% " "

Mouse tests

The experiments are to be done in duplicate - on batches of 50 mice at Birmingham and 50 at the Chester Beatty Research Institute. This will necessitate 600 mice in each place, 50 for each of the four dilutions of the three oils. The experiments are to be standardised as in I(1).

Rabbit tests

These are also to be done in duplicate - at Birmingham and at the Chester Beatty Research Institute.

Male rabbits are to be used, white inbred strains if possible and three -quarters grown.

Each rabbit is to be painted on six sites, the same sites as in I,2(a).

36 rabbits are to be used and divided randomly into 12 groups of three rabbits. The 4 dilutions of each of the three oils are to be assigned to the groups and sites as follows:

S I T E S

Rabbits	I	II	III	IV	V	VI
1 - 3	I 1	I 1/4	D 1/4	D 1/16	D 1/64	D 1
4 - 6	I 1/16	I 1/64	D 1	D 1/64	D 1/16	D 1/4
7 - 9	F 1/4	F 1	D 1/64	D 1	D 1/4	D 1/16
10 - 12	F 1/64	F 1/16	D 1/16	D 1/4	D 1	D 1/64
13 - 15	F 1	F 1/4	I 1/4	I 1/16	I 1/64	I 1
16 - 18	F 1/16	F 1/64	I 1/64	I 1	I 1/4	I 1/16
19 - 21	D 1	D 1/4	I 1/16	I 1/4	I 1	I 1/64
22 - 24	D 1/16	D 1/64	I 1	I 1/64	I 1/16	I 1/4
25 - 27	D 1/4	D 1	F 1/4	F 1/16	F 1/64	F 1
28 - 30	D 1/64	D 1/16	F 1/16	F 1/4	F 1	F 1/64
31 - 33	I 1/4	I 1	F 1	F 1/64	F 1/16	F 1/4
34 - 36	I 1/64	I 1/16	F 1/64	F 1	F 1/4	F 1/16

Dates of appearances of tumours and deaths to be recorded. The experiments to be continued for 12 months.

COPY

APPENDIX II

(REPORT OF THE THORNTON RESEARCH CENTRE (C 3.2/50, 7th MARCH, 1950)

CRUDE OILS - Job No. 224. Distillation of crude oil for the Institute of Petroleum

Requested by: S.R. & M. Co. Technological Department Memo. No. A.T.N./IP/7L, dated 25.2.49.

Summary

One ton each of Kuwait, Oklahoma Bright, and Laguinillas crude oils have been distilled to give four overhead fractions and one residue. Cracking was avoided during the distillation by maintaining the temperature of the liquid below 250° C and reducing the effective pressure by the introduction of steam.

Discussion

1. Distillation unit

The unit employed for the distillation is illustrated diagrammatically in Fig. 1. For steam distillation, saturated steam at 30 psig was injected into the base of the vessel V1, through a perforated coil. The rate of low of steam into the vessel was measured by a Foxboro orifice-type flow meter. The crude oil is distilled from V1 through condenser C1 to either of two primary receivers V2 and V3. Uncondensed vapour then passes through condenser C2 and V4. A 2 in. mild-steel line passes from this vessel to the vacuum pump.

2. Operation

Prior to the distillation the system was thoroughly cleaned, V1 was initially cleaned by hand and then a light kerosine was distilled from V1 to V2, V3 and V4 to flush out contaminants. A long period of steaming then followed to remove the hydrocarbons. This procedure of cleaning with kerosine and steam was carried out before the distillation of each of the three crude oils.

The crude oil was charged to the distillation vessel V1 via a line entering the vessel at the base. In order to avoid contamination of the crudes which might occur when using reciprocating pumps, charging was carried out by suction, the vacuum pump in the system being utilised for this purpose. A charge of ca 350 kg. was distilled per batch, so that the distillation of three batches was necessary to obtain the required amount of crude oil distillate.

After charging, the vessel V4 was vented to atmosphere and the crude oil in V1 heated to a maximum temperature of 240° C with stirring. The temperature of the oil entering and leaving V1 jacket was between 245° and 250° C. (During this stage care was required to avoid bumping, especially with the Laguinillas crude, which contained quite large amounts of water.)

The distillate was collected in V2 until the rate of distillation had decreased. At this point the vent at V4 was closed and the pressure slowly reduced to 150 mm Hg, the distillate being collected in V3. The two fractions so obtained were bulked to give fraction 1. The pressure was again reduced (to 100 mm Hg) and steam introduced to a maximum rate of 30 lb./hr. while three further fractions were collected. To simplify the operation, the volumes to be expected for these last three fractions were determined prior to the actual distillation by a laboratory distillation of the crude oil. Cut points in the plant distillation were then taken when the predetermined volumes had been distilled.

3. Drying and storing

After the distillations the corresponding fractions were bulked and allowed to settle. The water layer was removed and the oil partially dried by filtering slowly through cotton wool pads. This method was used to avoid unnecessary contamination by chemical driers. The dry fractions were then stored in new 40-gallon mild-steel drums. The residues from the distillations were dropped, while still hot, from the distillation vessel into new 40-gallong mild-steel open topped drums.

Results

The yields of each fraction, and simple physical characteristics of Kuwait, Oklahoma Bright and Laguinillas crude distillates are given below in Tables 1 to 3. It is interesting to note that in the high-sulphur-content Kuwait crude no hydrogen sulphide or sulphur dioxide was evolved during the distillation of fractions 2, 3, and 4. This is a good indication that no cracking occurred during the distillation. The relatively high losses were due primarily to losses in handling during drying, but in the case of Laguinillas crude the high water content considerably reduced the percent weight of oil distillate.

APPENDIX II

Table 1.

Kuwait crude

		Crude	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Residue
Weight	kg	1018	267	99	80	60	445
	%w	93.9%	26.3	9.8	7.9	5.9	43.0
Distillation range	I.P.method IP.28/42						
I.B.P.		39	44	167	284	330	-
5%		82	71	231	303	345	-
10%		117	81	242	303	350	-
20%		172	99	251	314	357	-
30%		225	116	258	318	361	-
40%		285	130	263	323	365	-
50%		332	142	273	327	368	-
60%		348	160	280	333	372	-
70%		355	180	290	340	378	-
80%		372	202	303	349	384	-
90%		390	321	319	363	393	-
95%		Cracked	259	339	375	-	-
F.B.P.		"	279	350	396	400	-
Recovery %		90.5	98	98	99	98	-
Residue %		9.5	1.0	1.5	1.0	2	-
Loss %		nil	1.0	0.5	nil	nil	-
Viscosity kinematic cS	IP.71/47T	26.0/70°F	0.95/70°F	4.53/70°F	14.0/70°F	9.26/140°F	1300/140°F
Redwood I sec	IP.70/46	108/70°F	28/70°F	37/70°F	65/70°F	50/140°F	5000/140°F
Flash point Abel closed cup °F	IP.33/44	60	50	146			
Pensky-Marten closed cup °F	IP.34/47				255	345	ca 450

* These figures have been amended to the correct proportions

APPENDIX II

Table 2

Oklahoma Bright crude

		Crude	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Residue
Weight	kg	1250	495	119	103	91	402
	%w	96.5	39.5	9.5	8.2	7.3	32.0
Distillation range	I.B.P method IP.28/42						
I.B.P.		40	44	211	248	296	-
5 %		60	69	226	280	309	-
10 %		102	81	231	285	315	-
20 %		140	100	237	290	320	-
30 %		175	117	245	294	327	-
40 %		230	130	250	299	332	-
50 %		271	144	255	305	338	-
60 %		311	164	264	311	344	-
70 %		350	181	273	319	350	-
80 %		354	204	284	329	359	-
90 %		364	233	303	346	375	-
95 %		370	268	323	361	391	-
F.B.P.		372	290	343	373	400	-
Recovery %		97.5	98	98	97.5	98.0	-
Residue %		2.5	1.0	1.5	2.0	2.0	-
Loss %		nil	1.0	0.5	0.5	nil	-
Viscosity kinematic cS	IP.71/47T	4.6/70°F	0.896/70°F	1.8/140°F	3.4/140°F	5.3/140°F	8.0/140°F
Redwood I sec	IP.70/46	38/70°F	27/70°F (calc)	31/140°F (calc)	35/140°F (calc)	39/140°F	335/140°F
Flash point Abel closed cup °F	IP.33/44	50	50				
Pensky-Marten closed cup °F	IP.34/47			185	250	285	420

APPENDIX II

Table 3

Laguinillas crude

Weight	kg %w	Crude	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Residue
		1120 92.0	92.5 8.2	94.8 8.4	58.8 5.3	51.4 4.6	734.8 65.5
Distillation range	I.B.P. method IP.28/42						
I.B.P.		100	97	223	262	317	-
5 %		200	126	248	301	338	-
10 %		262	139	251	310	343	-
20 %		318	153	261	317	350	-
30 %		334	166	269	322	354	-
40 %		345	181	277	329	360	-
50 %		350	195	287	336	365	-
60 %		368	209	294	342	370	-
70 %		384	222	302	352	376	-
80 %		Cracking	239	317	363	384	-
90 %		"	265	335	378	Cracked	-
95 %		"	291	351	392	"	-
F.B.P.		"	300	359	397	"	-
Recovery %		90	98	97	98	93	-
Residue %		3.0	2.0	3.0	3.0	7.0	-
Loss %		nil	nil	nil	nil	nil	-
Viscosity kinematic cS	IP.71/47T	165/140°F	1.605/70°F	2.9/140°F	7.3/140°F	16.7/140°F	-
Redwood I sec	IP.70/46	668/140°F	30/70°F	33/140°F	45/140°F	74/140°F	20,000/140 (calc.)
Flash point Abel closed cup °F	IP.33/44	50	50				
Pensky-Marten closed cup °F	IP.34/47			214	270	350	460

A Parr
J.N.L. Thomas

D. VICKERY

for Director of Research
Thornton Research Centre

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

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January 11, 1957

Mr. D. V. Stroop
American Petroleum Institute
50 West 50th Street
New York 17, New York

Dear Mr. Stroop:

I would greatly appreciate it if you would have the attached duplicated and distributed to the members of the Subcommittee on Carcinogenicity and to Dr. A. W. Horton of the Kettering Laboratories.

It is my understanding that the British are somewhat put out that they have not received anything from us in the past several years. I suggest that the subcommittee give serious thought as to what they are going to send the British so that we can discuss it at the next meeting of our subcommittee.

In addition, Dr. Newquist has suggested that perhaps Dr. Horton ought to summarize this, and all past reports received from the British. This occurs to me to be a good idea. In the event that Dr. Horton does not have some of the previous information received from England, if he would let me know I would be happy to see that he is brought up to date on anything that he may be lacking.

Very truly yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
Attach. - Six reports of the Committee on the Carcinogenic Action of Mineral Oils, Medical Research Council, London, attached to ltr to REE from Dr. Newquist, 1/2/57.

Copy From D. V. Stroop For Information Of And Consideration By Members Of The Subcommittee On Carcinogenicity And Dr. A. W. Horton 1/14/57

MEDICAL RESEARCH COUNCIL

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(Members of Committee)

MRC.56/963
CAMO. Ag.21

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

AGENDA

for the Twenty first meeting to be held
at 38 Old Queen Street, Westminster, S.W.1,
on Tuesday, 18th December, 1956 at 2.30 p.m.

1. Minutes of the previous meeting (MRC.56/727, CAMO.Min.20 - already circulated)

2. Progress Reports:-

(a) No.XVII, November 1956 (Birmingham), by Dr. D.L. Woodhouse, (MRC.56/946, CAMO.56/9 - Enclosure A).

(b) The 1955-56 Experiments with Mice at Leeds, by Dr. J.O. Irwin, (MRC.56/965, CAMO.56/12 - Enclosure B).

(c) Report by Dr. W. Carruthers, Dr. J.W. Cook and Mr. A.G. Douglas, (MRC.56/958, CAMO.56/11 - Enclosure C).

3. Preparation of Kuwait fractions for 1957 to 1958 tests.

As picrate treatment on a large scale has proved impracticable Professor Morton will suggest preparation of fractions by chromatography on alumina from KX5-6.

A Note by Professor Morton is enclosed (MRC.56/956, CAMO.56/10 - Enclosure D).

4. Proposal for including a single specimen of cutting oil, reported to be non-carcinogenic in the Birmingham tests.

Manchester Oil Refineries have prepared an oil which, according to the Twort Formula is non-carcinogenic, for Messrs. Ferranti, a big user of cutting oils. This oil satisfies all the requirements from the technical point of view, and it is now suggested that it might be tested in the laboratory to see if it is in fact relatively non-carcinogenic as far as animals are concerned. If it were so, then it could be, and perhaps should be, recommended for wider use as a cutting oil.

5. Any other business.

6. Date of next meeting.

MEDICAL RESEARCH COUNCIL

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MRC.56/94
CAMO.56/9

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Progress Report No. XVII , November, 1956 (Birmingham)

by

D.L. Woodhouse

The fractions listed in Table I were commenced on 28th February and have been applied for 37 weeks at the time of the report, when the tumours indicated were observed. Fuller details will be submitted at the end of the 52 week period, the present report being intended as a preliminary guide for future work.

Table I

Rabbit Tests

Fraction		Tumours
A)	K_4S_{6-8} Ari V (53 ml.)	1
B)	K_4S_{6-8} Ari W (53 ml.)	0
C)	K_4S_{6-8} Ari X (53 ml.)	0
D)	K_4S_{6-8} Ari Y (53 ml.)	0
E)	K_4S_{6-8} Ari Z (residue) (53 ml.)	1
F)	K_4S_{6-8} Arii X (15 ml.)	2
G)	K_4S_{6-8} Arii Y (15 ml.)	0
H)	K_4S_{6-8} Arii Z (residue) (15 ml.)	0
I)	K_4S_{6-8} Ariii X (11 ml.)	0
J)	K_4S_{6-8} Ariii Y (11 ml.)	0
K)	K_4S_{6-8} Ariii Z (residue) (11 ml.)	0
L)	K_4S_{6-8} Ari (original pet. ether eluate) (132 ml.)	0
M)	K_4S_{6-8} Arii (original benzene eluate) (23 ml.)	0
N)	K_4S_{6-8} Ariii (original methanol eluate) (17 ml.)	0
O)	KX_{2-4} (B.range 355-370 C) (300 ml.)	3
P)	KX_{5-6} (B.range 370-380 C) (300 ml.)	5
Q)	KX_{7-8} (B.range 380-390 C) (300 ml.)	3
R)	$KX_{Residue}$ (Boiling above 390 C) (300 ml.)	8
S)	K_4S_8 (original Stedman cut as standard) (300 ml.)	2
T)	D(2) Standard as agreed	13
U)	D($\frac{1}{2}$) Standard as agreed	8

See CAMO. 56/7 page 1.

Table II

Mouse Tests

Fraction.	Tumours
C) K_4S_{6-8} Arii Y	1
H) K_4S_{6-8} Arii Z	1
O) KX_{2-4}	1
P) KX_{5-6}	1
Q) KX_{7-8}	1
T) D(2)	35
U) D($\frac{1}{2}$)	25

All other fractions have produced no tumours.

Notes

1) For comparison of fractions O, P, Q with K_4S_2 to K_4S_8 see Birmingham Report No. XII.

K_4S_2	= 6 tumours	In rabbits all at 37 weeks.
K_4S_3	= 6 tumours	
K_4S_4	= 7 tumours	
K_4S_5	= 4 tumours	
K_4S_6	= 5 tumours	
K_4S_7	= 3 tumours	
K_4S_8	= 2 tumours	

2) Compare R with K_4S_9 = 3 tumours at 37 weeks (Birmingham Progress Report XVI).

3) Compare S = K_4S_8 with results in Birmingham Progress Reports XI and XII, i.e. 2 tumours at 37 weeks, 3 at 44 weeks and 6 at 52 weeks.

4) In 1954 - 55 (Birmingham Progress Report XVI)

Standard D(2) gave 15 tumours at 37 weeks
Standard D($\frac{1}{2}$) " 2 " " 37 "

In 1953 - 54 (Report XII)

Standard D(2) gave 10 tumours at 37 weeks
Standard D($\frac{1}{2}$) " 6 " " 37 "

MEDICAL RESEARCH COUNCIL

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MRC.56/965
CAMO.56/12

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

The 1955-56 Experiments with Mice at Leeds

by

J. O. Irwin

The results for the Standards are given in Table 1, and for the Fractions in Table 2.

The Standards

The responses to $D\frac{1}{2}$ were in good agreement with those obtained in previous years. In Table 1 these responses are compared with the results of the three experiments carried out at Birmingham in 1952-3, 1953-4 and 1954-5 and that carried out at Leeds in 1954-5.

The response to D_2 however seems to have been anomalous. The total number of tumours was 32 against an average of 38 in the Birmingham experience, but tumours occurred a great deal later, as shown by the expectation of tumourless life and date when 10% of survivors had tumours.

The Fractions

The Fractions tested were the eight Laguinillas L_4S_1A - L_4S_8A and the residue L_4S_R . In Table 2 these are compared with the corresponding Kuwait fractions tested at Birmingham in 1953-4.

With one exception to L fractions all gave a higher response than the corresponding K fractions. The difference was statistically significant for L_4S_5A, L_4S_6A, L_4S_7A, L_4S_8A, also these four fractions gave a higher response than the other L fractions. The residue L_4S_R gave a positive response while K_4S_R did not.

In view of the anomalous response to the standard these results should be interpreted with some caution. However, $D\frac{1}{2}$ at Leeds in 1955-6 gave a lower response than D_2 at Birmingham in 1953-4 and D_2 at Leeds gave a much lower response than D_2 at Birmingham in the same year. This suggests that the Laguinillas really were more potent than the corresponding Kuwait fractions, for if they were compared relative to the standards tested at the same time, their relative difference would be enhanced.

Table 1. Results from Standards in Mice Experiments (Leeds 1955-6)

Fractions		Expectation of Tumourless Life (Limited to 52 weeks)	Date when 10% of Survivors had Tumours	Total number of Tumours
Leeds Results (1955-6)				
D_2		32.5 ± 1.0	26.1 ± 0.4	32
$D_{\frac{1}{2}}$		23.3 ± 0.9	17.3 ± 0.6	42
Leeds Results (1954-5)				
D_2		16.1 ± 1.8	10.1 ± 0.5	41
$D_{\frac{1}{2}}$		18.8 ± 0.9	12.2 ± 0.2	44
Birmingham Results				
D_2	1952-3	13.9 ± 0.4	11.4 ± 0.8	38
	3-4	12.9 ± 0.6	8.7 ± 0.4	40
	4-5	14.4 ± 2.0	6.6 ± 0.2	36
	Mean	13.7 ± 1.2	8.9 ± 0.5	38
$D_{\frac{1}{2}}$	1952-3	24.8 ± 1.6	17.1 ± 0.6	25
	3-4	18.1 ± 0.8	10.3 ± 0.6	45
	4-5	17.9 ± 0.8	11.7 ± 0.3	43
	Mean	20.3 ± 1.1	13.0 ± 0.5	38

Table 2. Results from Fractions in Mice Experiments
Leeds (1955-6) Laguinillas and Birmingham (1953-4) Kuwait.

Fractions	Expectation of Tumourless Life (Limited to 52 weeks)	Date when 10% of Survivors had Tumours	Total number of Tumours
L_4S_1A	48.0 ± 2.0	29.4 ± 2.9	4
K_4S_1A	50.1 ± 0.6	not reached	2
L_4S_2A	49.5 ± 1.2	32.7 ± 3.8	4
K_4S_2A	52	not reached	0
L_4S_3A	52	not reached	0
K_4S_3A	51.2 ± 0.6	not reached	3
L_4S_4A	48.2 ± 2.1	not reached	3
K_4S_4A	49.6 ± 1.3	not reached	4
L_4S_5A	42.2 ± 3.9	27.7 ± 0.6	4
K_4S_5A	51.2 ± 0.6	not reached	2
L_4S_6A	43.2 ± 3.0	19.7 ± 3.5	7
K_4S_6A	50.7 ± 1.0	46.3 ± 4.0	2
L_4S_7A	39.7 ± 4.1	17.6 ± 0.6	6
K_4S_7A	50.4 ± 1.0	43.9 ± 0.6	3
L_4S_8A	36.8 ± 4.0	17.7 ± 0.4	8
K_4S_8A	47.3 ± 1.6	28.6 ± 2.3	8
L_4S_R	46.5 ± 1.7	24.3 ± 2.3	3
K_4S_R	52	not reached	0

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MRC.56/958
CAMO.56/11

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

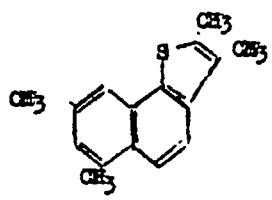
Report by Dr. W. Carruthers, Dr. J.W. Cook
and Mr. A.G. Douglas.

We are continuing with our investigation of fractions produced from the Kuwait Oil.

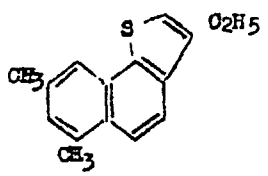
The distillates obtained by fractional distillation of the petroleum-ether eluates of picrate forming fractions have yielded a number of crystalline substances by crystallisation of molecular complexes and chromatography. Difficulty has arisen in purifying these substances, because of the small amounts available, but the ultra-violet absorption spectra of the crude materials shows that they belong to the class of phenanthrenes or of dibenzothiophenes. Two compounds have been obtained homogeneous; one is a tri- or tetra-methyldibenzo-thiophene, and the other is a tetra-methylphenanthrene.

One of the methanol eluates (K_4S_7bApiv) obtained from the Kuwait fraction b.p. 382½-385° as outlined in the last Report, has yielded a crystalline compound, m.p. 179-181°. The ultra-violet absorption spectrum and elementary analysis of this substance indicate that it is a penta- or hexa-methylcarbazole, or the equivalent.

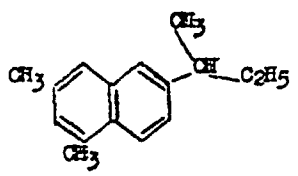
The compound $C_{16}H_{16}S$ isolated before from fraction K_4S_6bAp (see CAMO.54/4) has now been identified as 3-ethyl-6:8-dimethyl-naphtho (1:2-b) thiophene (II) (Ring-index nomenclature).



(I)



(II)



(III)

As outlined in previous Reports, the basic 6:7-benzothio-naphthen ring structure of the compound was shown by its ultra-violet absorption spectrum, and desulphurisation with Raney nickel afforded a naphthalene hydrocarbon $C_{16}H_{20}$. This has now been identified by synthesis as 1:3-dimethyl-6-sec-butylnaphthalene (III). Two possible structures (I and II) thus remained for the parent sulphur compound. A decision was made in favour of II on the basis of the C-methyl analysis which showed the presence of only 3 C-methyl groups, as expected for (II). Compound I would have shown 4 such groups.

6th December 1956

MEDICAL RESEARCH COUNCIL

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MRC.56/954
CAMO.56/10

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Suggested Programme of Biological Tests at Birmingham

1957-58

Note by Prof. F. Morton

The following programme of tests for Dr. Woodhouse was discussed at Birmingham on 14 November by Professor Squire, Dr. Woodhouse, Dr. King and Professor Morton (Dr. Carruthers was unable to attend).

The reason for the modification in the original scheme, which was to prepare samples by the methods of separation developed by Dr. Carruthers, is our inability to handle large quantities of picric acid. Various industrial concerns have been approached but are not prepared to undertake this work.

We propose therefore to undertake the separation by liquid phase chromatography using alumina, following the publication by Mair et al (A.C.S. Pet.Div. Sept. 1956) which shows the same separation into polynuclear, di-nuclear and mono-nuclear aromatics is possible. The use of such technique on a large scale cannot be undertaken in our present laboratory (for safety reasons) and we therefore suggest a preliminary separation of the KX_{5-6} fractions (based on the interim report of Dr. Woodhouse) to provide samples for the 1957-58 series at Birmingham and some material for preliminary work by Dr. Carruthers.

The proposal involves firstly the acetone-water extraction of KX_{5-6}, to produce a more highly aromatic extract and a raffinate (KX_{5-6} P). The extract KX_{5-6} A will then be chromatographed on alumina and the separation followed by refractive index measurements on the elutrates. By this means we expect to obtain some indication during the preparation of the Petroleum Ether, Benzene and Methanol elutrates of differences in the hydrocarbons used in each elutrate. Further separation of the Petroleum Ether and Benzene elutrates will be carried out if the refractive index measurements indicate that some further separation is possible. The Methanol elutrate will be studied only if biological results of the 1956 series indicate some activity. To check on possible loss of active components during chromatography the elutrates will be reblended in aliquot proportions and the blend included in the test samples.

In addition it is proposed to include:

1. The usual standards (D_2 and $D_{\frac{1}{2}}$).
2. KX_9, a sample prepared by distilling the KX residues in a manner similar to the preparation of K_4S_9.
3. At Professor Squire's request a cutting oil which has been offered as being non-potent.

The list of samples will not be completed until the examination of the elutrates has been discussed with Dr. Carruthers and Professor Squires, i.e. samples 12 - 21 may be prepared by further separation of KX_{5-6} Ai and KX_{5-6} Aii, or alternatively Dr. Carruthers may wish to include some individual hydrocarbons in this series.

Sample No.	Description	
1	D2 Standard	
2	$D\frac{1}{2}$ "	
3	Cutting oil	
4	KX_9	
5	KX_{5-6}	(To repeat 1956 tests)
6	KX_{5-6} A	(Acetone extract)
7	KX_{5-6} P	(Raffinate)
8	KX_{5-6} Ai	(Petroleum Ether elutrate)
9	KX_{5-6} Aii	(Benzene elutrate)
10	KX_{5-6} Aiii	(Methanol elutrate)
11	Blend of KX_{5-6} Ai, ii and iii	

Nos.12 - 21 To be decided after examination of analysis of elutrates.

Standard strength of all KX_{5-6} samples to be equal to KX_{5-6} A. Thus, the KX_{5-6} Ai etc. will be diluted with liquid paraffin to the same volume as the KX_{5-6} A from which they are prepared.

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COMMITTEE ON THE CARCINOGENIC ACTION OF ETHERAL OILS

meeting to be held at 2.30 p.m. on Tuesday the 18th December 1956, at 38 Old Queen Street, S.W.1.

The attached report (MRC.56/969, CAN.O.56/13), is for discussion under item 2(b) of the Agenda.

11th December 1956.

R.C.MORTON.

MEDICAL RESEARCH COUNCIL

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MRC.56/969
CAMO.56/13

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Statistical Analysis of 1955-6 Experiments with Rabbits at Leeds

by J. O. Irwin

An experiment with 11 fractions was carried out. The fractions were L_4S_1A - L_4S_8A the residue L_4SP together with the two standards D_2 and $D\frac{1}{2}$.

The design of the experiment was the 11 x 5 Youden square shown in Table 1. There are 11 groups of 5 rabbits. In each group each rabbit was painted with the same 5 fractions one on each of the sites I, II, III, IV, VII. Each of the 11 sub-fractions was painted on each site in one and only one group. Each fraction was used in exactly five groups and every pair of fractions in just two groups.

The percentage of each group of animals which got tumours on each site was calculated. In this experiment there was an unusual number of tumourless deaths before the end of the year - 17 out of 55. Accordingly an allowance was made for them. The number of animal years of exposure was calculated for each group. For example in the sixth group there was one tumourless death 11 weeks from the beginning of the experiment. The number of animal weeks of exposure was therefore 11 + (4 x 52) = 219 and 219/52 = 4.2. This was rounded off to the nearest unit i.e. 4 and the three tumours on site I were considered to have occurred in 4 animals exposed for a full year. The percentage was therefore taken to be 75% not 60%.

The method of analysis was to transform percentages to degrees by means of the angular transformation and then perform an analysis of variance of the results.

Table 2 shows the mean value of the transformed variables and the probabilities of getting a tumour, estimated for each fraction and standard. The corresponding values of the K series tested at Birmingham in 1953-4 are shown for comparison.

The Standards

The results for the standards are compared below with those of two previous years.

	Leeds 1955-6	Leeds 1954-5	Birmingham 1953-4
D_2	.627 (51.18)	.644 (52.27)	.643 (52.19)
$D\frac{1}{2}$	.521 (46.02)	.245 (31.84)	.156 (26.63)

The figure shown is the estimated probability of getting a tumour. (The transformed variable is shown in brackets. The standard errors are 4.2, 4.7, 5.0 respectively for 1955-6, 1954-5, 1953-4 respectively.) The result for $D\frac{1}{2}$ seems unusually high. For D_2 the agreement is good.

The fractions

L_4S_1A shows a rather low carcinogenicity, so does L_4SP, though the value for the latter is higher than for K_4SP; (the difference just about reaches the 5% level of significance). Otherwise there is little difference between the response of the different L fractions. The L fractions give a somewhat higher response than the corresponding K fractions and this result agrees with that obtained for the mice.

Analysis of Variance

The analysis of variance is shown in Table 3. There are significant differences between groups of rabbits, and the site differences are remarkably large. The observed percentage of tumours on the various sites is shown in Table 4. The highest incidence is again on sites I and II (ears) and VII (middle of the back nearest ears).

Table 1

Design for Experiment with Rabbits at Leeds (1955-6)

Rabbits	I	II	III	IV	VII
1 - 5	c	d	a	b	e
6 - 10	f	j	g	a	c
11 - 15	a	f	i - h		b
16 - 20	e	h	j	k	a
21 - 25	b	c	h	g	k
26 - 30	i	b	e	j	g
31 - 35	g	a	k	i	d
36 - 40	h	g	d	e	f
41 - 45	d	k	b	f	j
46 - 50	k	e	f	c	i
51 - 55	j	i	c	d	h

L_4S_1A	= c	a	= L_4S_2A
L_4S_2A	= a	b	= L_4S_6A
L_4S_3A	= j	c	= L_4S_1A
L_4S_4A	= h	d	= D_2
L_4S_5A	= e	e	= L_4S_5A
L_4S_6A	= b	f	= $D_{\frac{1}{2}}$
L_4S_7A	= i	g	= L_4SP
L_4S_8A	= k	h	= L_4S_4A
L_4SP	= g	i	= L_4S_7A
D_2	= d	j	= L_4S_3A
$D_{\frac{1}{2}}$	= f	k	= L_4S_8A

Table 2

Experiment with Rabbits at Leeds 1955-6

Two Standards and eleven derived fractions

Mean Values corrected for Group differences

Leeds 1955-6					Birmingham 1953-4				
Fraction or Standard	Transformed Variable	Probability of Tumour	Total Number of Tumours		Fraction or Standard	Transformed Variable	Probability of Tumour	Total Number of Tumours (out of 25)	
L_4S_1A	34.96	.299	8/22	36%	K_4S_1A	43.68	.474	10	40%
L_4S_2A	52.03	.648	10/17	59%	K_4S_2A	25.67	.141	5	20%
L_4S_3A	51.98	.642	14/21	67%	K_4S_3A	41.88	.438	11	44%
L_4S_4A	47.16	.546	10/18	56%	K_4S_4A	51.57	.631	14	56%
L_4S_5A	50.08	.606	11/19	58%	K_4S_5A	48.14	.563	14	56%
L_4S_6A	50.65	.618	11/18	61%	K_4S_6A	52.77	.654	16	64%
L_4S_7A	52.88	.664	13/18	72%	K_4S_7A	38 35	.366	10	40%
L_4S_8A	51.58	.636	12/18	67%	K_4S_8A	49.35	.587	13	52%
L_4SP	34.52	.288	5/20	25%	K_4SP	21.28	.077	2	8%
D_2	51.18	.627	14/20	70%	D_2	52.19	.643	17	68%
$D_{\frac{1}{2}}$	46.02	.521	9/19	47%	$D_{\frac{1}{2}}$	26.63	.156	6	24%
S.E. one mean	4.21					4.97			
Difference between two means	5.95					7.03			

Table 3

Experiment with Rabbits at Leeds 1955-6

Analysis of variance on angular transformation of proportion of tumours

	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	
Sites	7478.43	4	1869.61	23.23	($P < .001$)
Groups of Rabbits	3119.77	10	311.98	3.88	($.01 < P < .001$)
Fractions	2565.98	10	256.60		
Error	2414.84	30	80.49		
	15579.02	54			

Table 4

Experiment with Rabbits at Leeds 1955-6

Number and Percentage of Tumours on the five different sites

Site	Number of Tumours	% Tumours
I	33/42	79%
II	33/42	79%
III	12/42	29%
IV	13/42	31%
VII	26/42	62%

Copy From D. V. Stroop For Information Of Members Of Medical Advisory Committee and Kettering Laboratory 2/13/56

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P. O. Box 121, Linden, N. J.

MEDICAL RESEARCH DIVISION

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BERTRAND E. BENNISON, M.D.
CHIEF CLINICAL RESEARCH PHYSICIAN

February 9, 1956

Mr. D. V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

The attached information has been received through Dr. Newquist's office from the Medical Research Council. It would probably be of interest to all members of the Medical Advisory Committee, and the group at Kettering Laboratory. I would greatly appreciate it if you would arrange for its duplication and distribution.

Sincerely yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:mak

Att.-Ltr. MRC.55/1016
CAMO.56/1
to Com. on the Carcinogenic
Action of Mineral Oils.

MEMORANDUM FROM A. E. DOOLEY

NEW YORK, N. Y. Feb. 3, 1956

TO MR. R. E. Eckardt

This report was received by Dr. Newquist yesterday from England. You may wish to have it distributed to the members and associates of the Medical Advisory Committee.

E.C.

MEDICAL RESEARCH DIVISION

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MRC.55/1016
CAMO.56/1

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Short-term Skin Tests for Carcinogenicity
Carried out by Dr. William E. Smith

Following the meeting at which Dr. William E. Smith described his short-term tests for carcinogenicity, Dr. Woodhouse sent him 13 samples, without indicating what they were, and the results of Dr. Smith's tests are attached. Samples 3 and 13 were broken in transit and therefore were not tested. For convenience, a key identifying samples has been added to Dr. Smith's Table 1, (see MRC.55/1010, [illegible] 158 of the 19th meeting.

OBSERVATIONS UPON EARLY CHANGES IN MOUSE SKIN
FOLLOWING APPLICATION OF OILS RECEIVED FROM DR. D. L. WOODHOUSE.

TABLE 1. Enumeration of sebaceous glands after four daily applications of oils at 5% conc. in acetone.

Sample No.	Sebaceous glands per cm. (Individual mice)					Sebaceous glands per cm. (Average)	Predicted Carcinogenicity	Key
10	0	0	0	0.5	1	0.4	Strong	L_4
9	0	0	1	1	1.5	0.7	Strong	K_4
7	0	0	1.5	1.5	2.5	1.1	Strong	K_4S_9
2	5	5	6	8	12	7.2	Weak	L_2
12	8	8.5	8.5	10		8.8	Weak	L_5
1	2	8	13	13.5	16.5	10.6	Doubtful	K_5
4	10	10.5	11	12	16	11.9	None	O_4
6	10	12.5	12.5	15	18	13.4	None	Liquid Paraffin
5	10	17	19	23.5		17.4	None	Perylene 0.2% in L.P.
8	12	16.5	18	20	33.5	20	None	$D(\frac{1}{2})$
11	16	20	23	26	34.5	23.9	None	K_4S_5abAm

TABLE 2. Hair loss nine days after single application of undiluted oils

Sample No.	Hair loss (Individual mice)				Predicted Carcinogenicity
10	++++	++++	++++	+	Strong
9	+++	+++	++	++	Strong
12	+	+	+	+	Weak
2	+	+	0	0	Weak
1	+	0	0	0	Doubtful
4	+	0	0	0	Doubtful

N.B. No hair loss followed application of samples 5, 6, 7 or 8 or 11. These would be considered non-carcinogenic but note that Sample 7 gave a strongly positive sebacious gland test.

MEDICAL RESEARCH COUNCIL

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MRC.55/1010
CAMO. Min.19

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Minutes of the Nineteenth Meeting held at 26 Old Queen Street, London, S.W.1, on Tuesday, 25th October, 1955, at 11.0 a.m.

Present: Professor T. Ferguson (Chairman), Dr. J.W. Cook, Professor H.N. Green, Dr. I. Hieger, Dr. J.O. Irwin, Professor R.D. Passey, Professor J.R. Squire, Dr. D.L. Woodhouse and Mr. J.C.R. Hudson (Secretary).

Attended: Dr. R.C. Norton.

An apology for absence was received from Professor F. Morton.

156. Minutes

The following alterations were made to the Minutes of the Eighteenth Meeting:

i) Minute 150, line 3

"..... had identified 7, of which 3 were previously unknown" to read "..... had identified 7; 3 others were previously unknown."

ii) Line 4

"..... dibenzthiophene" to read "1:8 dimethyl dibenzthiophene."

The Minutes of the Eighteenth Meeting were then approved and signed.

157. Nomenclature (Minute 152)

(i) The memorandum (MRC.55/815, CAMO.55/8) prepared by Professor Squire and Professor Morton was received by the Committee and its recommendations approved.

Thus the following amendment to Minute 136 was accepted:

Paragraph 4, line 7:

DELETE "..... were split into aromatic (A) and raffinate (R) portions."

SUBSTITUTE "..... were split into aromatic (A) and paraffin raffinate (P) portions."

This amendment calls for the following amendments in the documents, listed below, prepared for the Committee since the formulation of Minute 136:

CAMO.55/2 and CAMO.55/6; for R read P
CAMO.55/14; CAMO.55/3 and CAMO.55/4; for M, P and R read m, p and r.

(ii) Dr. Woodhouse reported that he was preparing a survey of all the oil fractions from the 3 original oils indicating those which had been prepared, those which might be prepared, and those which have already been

the subject of investigation. The Committee felt that this would be a valuable document for them to have and Dr. Woodhouse agreed to complete it, to add an introductory paragraph explaining the basis of the nomenclature used (including the latest developments) and to submit this to the Secretary for circulation to the Committee.

158. New Rapid Screening Tests by Dr. W.E. Smith for Possible Carcinogens (Minute 155)

(i) Professor Squire reported that Dr. W.E. Smith had written to him to ask if it would be possible for the Council to cover the expenses of the testing of oil fractions supplied to him by Dr. Woodhouse if difficulty was found in finding these from other sources. The Committee agreed that the limited sum mentioned could be recommended as a justifiable expense to the Council if this proved necessary.

(ii) Dr. Smith had submitted a report on the result of his tests on the oil fractions supplied by Dr. Woodhouse, a copy of which has been circulated to the Committee as paper No. MRC.55/1016, CAMD.56/1 . As far as the oil fractions were concerned Dr. Smith's results from the short-term tests were in agreement with the long-term tests carried out by the Committee. However, sample no. 8 supplied to Dr. Smith had given a negative result in his short-term test although it was the 9:10 dimethyl 1:2 benzanthracene standard ($D\frac{1}{2}$) used by the Committee. This failure to identify the standard as a carcinogen led the Committee to doubt the general validity of Dr. Smith's test. It was also concluded that only the existing long-term test methods would suffice for the Committee's purposes at present.

It was agreed, however, that Professor Squire should write to Dr. Smith telling him of the success and failure of his tests and offering, on behalf of the Committee, to provide him with further blind samples should he wish to investigate the discrepancy in the first test. In this respect, the Committee suggested that if he asked for further samples they should include, in addition to the dimethylbenzanthracene standard, a second known carcinogen that was known to be less liable to photo-oxidation.

159. Progress Reports

A Progress Report by Dr. W. Carruthers and Dr. J.W. Cook (MRC.55/814, CAMD.55/9)

Dr. Carruthers explained that as Professor Morton had experienced some difficulty in preparing an aromatic extract of K_4S_9 this fraction had not been subjected to chromatographic separation as had been suggested at the previous meeting (Minute 151).

Dr. Cook reported that a paper had been published in "Nature", 176, 790 and that a second paper had been submitted for publication in the Journal of the Chemistry Society.

B Progress Report No.XV by Dr. D.L.Woodhouse and Professor J.R.Squire (MRC.55/804, CAMD.55/7)

There had been no significant changes in the numbers of tumours found within the two weeks since the Report was prepared. The results were in accordance with previous findings. From Table 1 it appeared that there might not be any significant difference between the number of tumours resulting from the picrate-forming fractions (p) and the tumours resulting from the residue (r). It was noted that in restoring the fractions to the concentrations found in the original oil samples the picrate-forming fractions had been more heavily diluted than the residues.

It was noted that Dr. Woodhouse's tests would be completed within a few weeks and it was agreed that he should send his results before the end of the year to Dr. Irwin for statistical analysis.

C.Progress Report by Professor H.N. Green (MRC.55/840, CAMO.55/10)

Professor Green said that he had little to report at this stage since the start of the investigation had been delayed due to limited accommodation and an epidemic among the rabbits.

160. Extension of Work at Leeds

Professor Green explained that his work on behalf of the Committee was bound to be limited by the amount of accommodation and technical assistance available. Financial support for the work would be required before it could be extended.

The Committee agreed that it was highly desirable that the animal investigations should be continued and extended at Leeds, not only as a means of checking the results obtained at Birmingham, but also in order to speed the work. For example, it was only at Leeds that Lagunillas oil fractions were being tested.

The Committee agreed that they would support an application by Professor Green to the Council for a grant in this respect.

161. Future Plans

(i) It was agreed that the new fractions to be tested should be those separated on the spinning band column by Professor Morton from each of the 3 pooled eluates prepared by Dr. Carruthers by chromatographic separation. At present, Professor Morton was carrying out a trial separation to determine how many fractions could be obtained from the 3 eluates.

(ii) It was necessary to determine whether the commercially prepared solvent extracted Kuwait fraction now in Professor Morton's hands produced sub-fractions similar to the K_4SA series already tested, since insufficient material derived from the original K_4 now remained. Accordingly, in the next year's experiments some of these commercial extracts (to be known as KE_6, KE_7 etc.) should be studied to determine their equivalence, under biological tests, to K_4S_6A, K_4S_7A etc.

(iii) The suggestion was made that some of the chemical compounds so far isolated by Dr. Carruthers might be tested biologically for carcinogenicity. However, it was agreed that these should not be tested until after the next series of tests, by which time more compounds might have been isolated and a larger supply of some compounds already isolated might be available.

It was agreed that the details of the fractions to be tested should be arranged between Professor Squire, Professor Morton and Dr. Carruthers with statistical advice from Dr. Irwin. Professor Squire agreed to send a copy of the finally agreed experimental procedure to the Secretary for circulation to the Committee.

162. Second Progress Report

It was suggested that as a means of assessing the progress of the research it would be useful to the Committee to prepare a second report as a continuation of the first report. It was agreed that this should be prepared early in 1956 with the hope that it would be ready, at least in draft, in time for the next meeting.

163. Date of Next Meeting

It was agreed that the next meeting should be held between mid-June and mid-July 1956, with a preference for a date as late as possible within that period.