THE SIGNIFICANCE OF DENSITY, REFRACTIVE INDEX AND VISCOSITY OF MINERAL OILS IN RELATION TO THE TYPE AND DEGREE OF ANIMAL REACTION

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From the Laboratories of the Manchester Committee on Cancer

(With 2 Graphs in the Text)

Our work during the last ten years in this connexion has embodied a varied assortment of experiments, but in this paper it is proposed to deal essentially with some of our more recent investigations on density measurement of oils recovered from the peritoneal cavity of animals after injection. It may be recalled that in our examination of the animal reaction to mineral oils we have adopted two major procedures, viz. (1) the painting of oil on the skin of the interscapular region of mice, and (2) the injection of oil into the peritoneal cavity of mice. Our main conclusions from the painting experiments were that the higher, within viscosity limits, the density, refractive index or refractivity of a mineral oil the more likely was it to prove to be carcinogenic, but that refractivity was on the whole a much more reliable guide than either density or index alone. Experiments showed that it was only with oils within a certain viscosity range that we were enabled to obtain tumours of the skin of our animals.

The alteration in the physical characteristics of a mineral oil injected into, and subsequently recovered from, the peritoneal cavity of an animal gives indications as to the toxicity of the said oil for the animal economy. In previous communications we have shown that there is a fall in the refractive index roughly proportional to the carcinogenic potency, but that the amount of fall may be greatly influenced by viscosity. Recent experiments have verified our previous findings, and in addition we have learnt that changes in the index are almost always accompanied by changes in the density. Active oils when recovered from the animal show a fall in density, the fall being such that there is usually a fall in the refractivity. The first 100 oils recovered 1 week after injection gave an average index and density fall of 0·0062 and 0·0072 respectively. The ratio of index fall to density fall, here 0·861 to 1, varies considerably with individual oils, although it does not vary greatly among distillates of crude mineral oils having changes of similar intensity. As far as recovered oils are concerned our index figures are more reliable than the density figures, as the amount of oil recovered from the animal (usually a mouse) is in favourable cases only about one-fifth of a cubic centimetre.

It was found that there is a tendency for oils which have a large index fall to have a relatively greater density fall, the index-density fall ratio being as a rule below the average. Thus as animals usually react relatively vigorously to skin applications or injections of oils having a high refractive index, such oils recovered after injection generally show an index-density fall ratio below the average. By arranging our oils in order of decreasing index-density fall ratio and determining correlations by the simple ranking method where perfect correlation equals 1, the following figures were obtained:

Index-density fall ratio and	refractive index fall	−0·313
,,	density fall	−0·594
,,	refractivity fall	−0·425
,,	original index	−0·174
,,	original density	−0·136
,,	original refractivity	−0·047

It may be mentioned here that among these oils the correlation of original index and original density is about 0·95, that of index fall and density fall of the recovered oils being about 0·90.

We have already seen that indications as to the probable carcinogenic potency of an oil are much better given by a consideration of the refractivity than of the index and density of the original oil; but with recovered oils things are reversed, density fall or index fall being a better guide than refractivity fall. Density determinations of the first 100 samples examined were not in all cases available for the recovered oils, but the following correlation values illustrate our point:

Carcinogenic potency and	index fall	0·758
,,	refractivity	0·709
,,	original index	0·272

A second set of correlations obtained with fifty oils were:

Carcinogenic potency and	density fall	0·563
,,	refractivity	0·552
,,	index fall	0·540
,,	refractivity fall	0·241

Thus density fall and index fall of the recovered oils give as good a correlation with carcinogenic potency as does refractivity of the original oil, but additional information can be obtained by comparing the physical characteristics of both the original and recovered oil. As an example of our meaning, we find that if a series of paraffinic base oils are compared with a series of naphthenic base oils of more or less similar carcinogenicity, the average refractivity of the original oils is definitely higher in the former group while the density and index fall of the recovered oils is on an average somewhat higher in the latter group.

The severity of the animal reaction to the injected oil is closely dependent on the density, index and viscosity of the oil, but for showing the relative importance of each factor by correlation methods random sampling, of course, does not give such clear-cut information as selective sampling, although in the latter case the deviations are mostly narrowed down. Thus the correlations found with 100 random samples of oils, both within and without the carcinogenic viscosity range, were:

Index fall and original index	−0·228
Index fall and kinematic viscosity	−0·521
Original index kinematic viscosity	−0·463

When, however, the 100 samples were divided into five grades of twenty samples, (*a*) according to kinematic viscosity, (*b*) according to index, and (*c*) according to index fall, the figures obtained were:

(*a*) Index fall and original index	0·746
(*b*) Index fall and kinematic viscosity	−0·801
(*c*) Original index kinematic viscosity	0·814

Thus, provided we know the viscosity of an oil, we have only to determine the refractive index to have a good idea of how the animal will respond to injection, and consequently to painting, of the particular sample under examination.

The fall in index and density from day to day

The reaction of the animal to the injected oil is a gradual process, taking, at least with most lubricating oils, many months before the residual oil remaining in the cavity is deprived, even approximately, of its activity; but for our standard test the oil is allowed to remain in the cavity for only 1 week. During the whole of the time there appears to be a progressive fall in index and density, the process being rapid at first and gradually subsiding from day to day, the index-density fall ratio usually remaining fairly constant for any particular oil. As the animal will not tolerate more than 0·5 c.c. of the average oil, and the residual oil free in the cavity tends daily to become less, it is difficult to obtain a complete set of density figures beyond a fortnight, but in most cases the index figures, obtained from a much smaller quantity of oil, give one

Table I. *Index and density fall in oil recovered from series of injected mice*

Oil 209	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fa[ll] / Density f[all]
Original oil	0·9203	1·5125	0·5569	—	—	—	—
Animal residue: 1 day	0·9162	1·5093	0·5559	0·0041	0·0032	0·0010	0·780
2 days	0·9146	1·5070	0·5543	0·0057	0·0055	0·0026	0·965
3 ,,	0·9128	1·5053	0·5536	0·0075	0·0072	0·0033	0·960
4 ,,	0·9106	1·5045	0·5540	0·0097	0·0080	0·0029	0·825
5 ,,	0·9099	1·5029	0·5527	0·0104	0·0096	0·0042	0·923
7 ,,	0·9087	1·5017	0·5521	0·0116	0·0108	0·0048	0·931
8 ,,	0·9068	1·5009	0·5524	0·0135	0·0116	0·0045	0·859
9 ,,	0·9108	1·5015	0·5506	0·0095	0·0110	0·0063	1·158
10 ,,	0·9092	1·4996	0·5495	0·0111	0·0129	0·0074	1·162
11 ,,	0·9051	1·4995	0·5519	0·0152	0·0130	0·0050	0·855
14 ,,	—	1·4992	—	—	0·0133	—	—
15 ,,	—	1·4982	—	—	0·0143	—	—
16 ,,	0·9020	1·4978	0·5519	0·0183	0·0147	0·0050	0·803
17 ,,	—	1·4974	—	—	0·0151	—	—
18 ,,	0·8978	1·4965	0·5530	0·0225	0·0160	0·0039	0·711
19 ,,	—	1·4972	—	—	0·0153	—	—
21 ,,	0·9017	1·4976	0·5518	0·0186	0·0149	0·0051	0·801
22 ,,	—	1·4961	—	—	0·0164	—	—
23 ,,	—	1·4956	—	—	0·0169	—	—
24 ,,	—	1·4948	—	—	0·0177	—	—

all the information required. A specimen result with a blend of Venezuelan oil and Mexican oil is given in Table I, the determinations of the physical properties being carried out, as in all other determinations referred to in this paper, at 25° C.

Animals are, as a rule, injected on a Monday, so that data for the 6th day are not usually available, and are procured only for special requirements. The average fall in the index of the first fifty samples of oils tested on a series of fifty groups of ten animals, one of each group being killed daily, is given below. Density measurements are not given as in many instances the quantity of oil available was insufficient for the purpose:

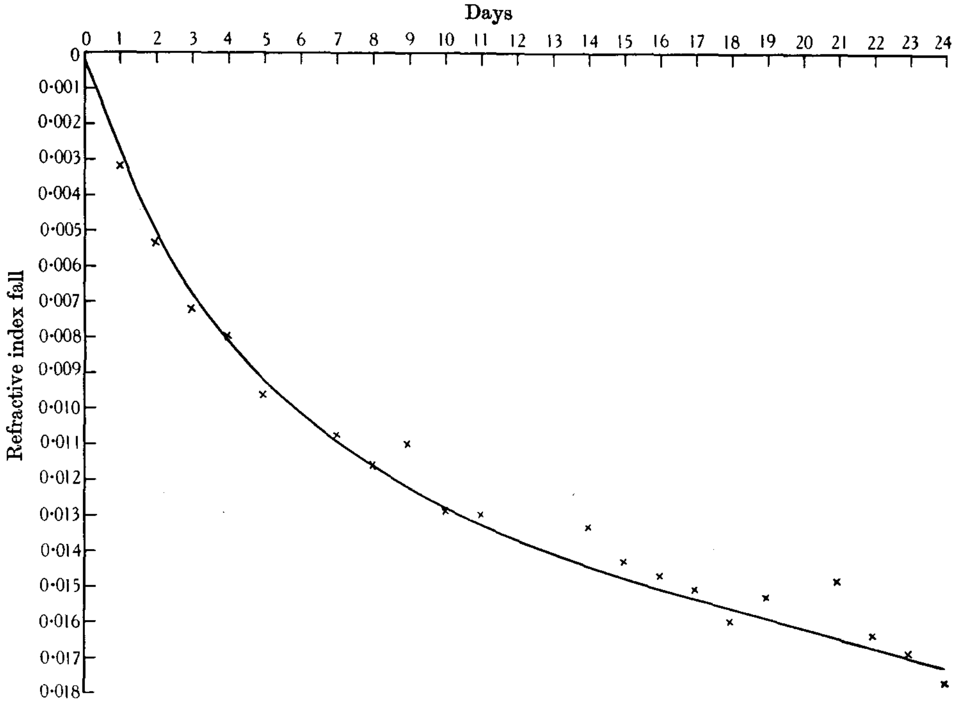
Day	Average refractive index fall: Per day	Average refractive index fall: Total	Percentage of total fall per day
1	0·00245	0·00245	26·98
2	0·00159	0·00404	17·51
3	0·00112	0·00516	12·33
4	0·00083	0·00599	9·14
5	0·00079	0·00678	8·70
*7	0·00110	0·00788	12·12
8	0·00051	0·00839	5·62
9	0·00050	0·00889	5·51
10	0·00019	0·00908	2·09

* 48 hours' interval.

THE FALL IN INDEX AND DENSITY OF OILS AFTER CHEMICAL AND PHYSICAL TREATMENT

It is quite evident that the change in the physical characteristics of an injected oil is brought about by the endeavour of the animal to effect detoxication. The means utilized to attain this result constitutes the main problem which the present experiments were designed to solve. In the first place the data available from an examination of oils recovered from the animal body were graphically depicted, and attempts were then made to imitate our graphs by treatment of the oil in the laboratory by various chemical means. In Graph 1, compiled from the data in Table I, is depicted the actual fall in the refractive index up to the 24th day after injection of the oil. A graph of the actual density fall may be superimposed for purposes of rapid appreciation of index-density fall ratio. Often, however, we are more concerned with the refractivity fall, in which case our figures are converted to a percentage basis, and we are enabled to compile Graph 2. Here we have the percentage fall of the index -1 and the percentage fall of the density, so that the percentage fall in refractivity is at once available for comparison with that of any other oil.

Sulphuric acid treatment, extraction with alcohol, oxygenation, and hydrogenation were among the processes tested, the preparation of the specimens having been carried out for us by our collaborator, Dr Bottomley. The alteration in the physical characteristics of oil 209 by treating with sulphuric acid is shown in Table II, and a comparison of the data here with those given in



Graph 1. Oil 209 recovered from a series of animals.

Table II. *Treatment of mineral oils with sulphuric acid*

Oil 209	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Original oil	0·9203	1·5125	0·5569	—	—	—	—
Mixed with 2 % acid	0·9171	1·5108	0·5570	0·0032	0·0017	+0·0001	0·531
,, 4 ,,	0·9170	1·5107	0·5569	0·0033	0·0018	0·0000	0·545
,, 6 ,,	0·9159	1·5100	0·5568	0·0044	0·0025	0·0001	0·568
,, 8 ,,	0·9134	1·5082	0·5563	0·0069	0·0043	0·0006	0·623
,, 10 ,,	0·9114	1·5069	0·5562	0·0089	0·0056	0·0007	0·629
,, 12 ,,	0·9089	1·5049	0·5555	0·0114	0·0076	0·0014	0·667
Shaken with 5 % acid	0·9126	1·5078	0·5564	0·0077	0·0047	0·0005	0·610
,, 10 ,,	0·9071	1·5037	0·5553	0·0132	0·0088	0·0016	0·667
,, 15 ,,	0·9019	1·5000	0·5544	0·0184	0·0125	0·0025	0·679
,, 20 ,,	0·8989	1·4976	0·5536	0·0214	0·0147	0·0033	0·687
,, 25 ,,	0·8948	1·4949	0·5531	0·0255	0·0176	0·0038	0·690
,, 35 ,,	0·8883	1·4899	0·5515	0·0320	0·0226	0·0054	0·706

Table I shows clearly differences between the animal reaction and the effect of the acid. In the latter case the ratio of index fall to density fall is definitely less than that given by the animal. Incidentally the effect of simple mixing and vigorous shaking of oil and acid on the amount of index and density fall is well illustrated in this table. It shows the importance of standardizing very carefully the procedure to be adopted. It is to be noted that as the acid treatment becomes more and more severe, there is, of course, a corresponding increase in the amount of both index and density fall, but unlike the effect of the animal where the two processes run parallel, when the oil is left in the animal for

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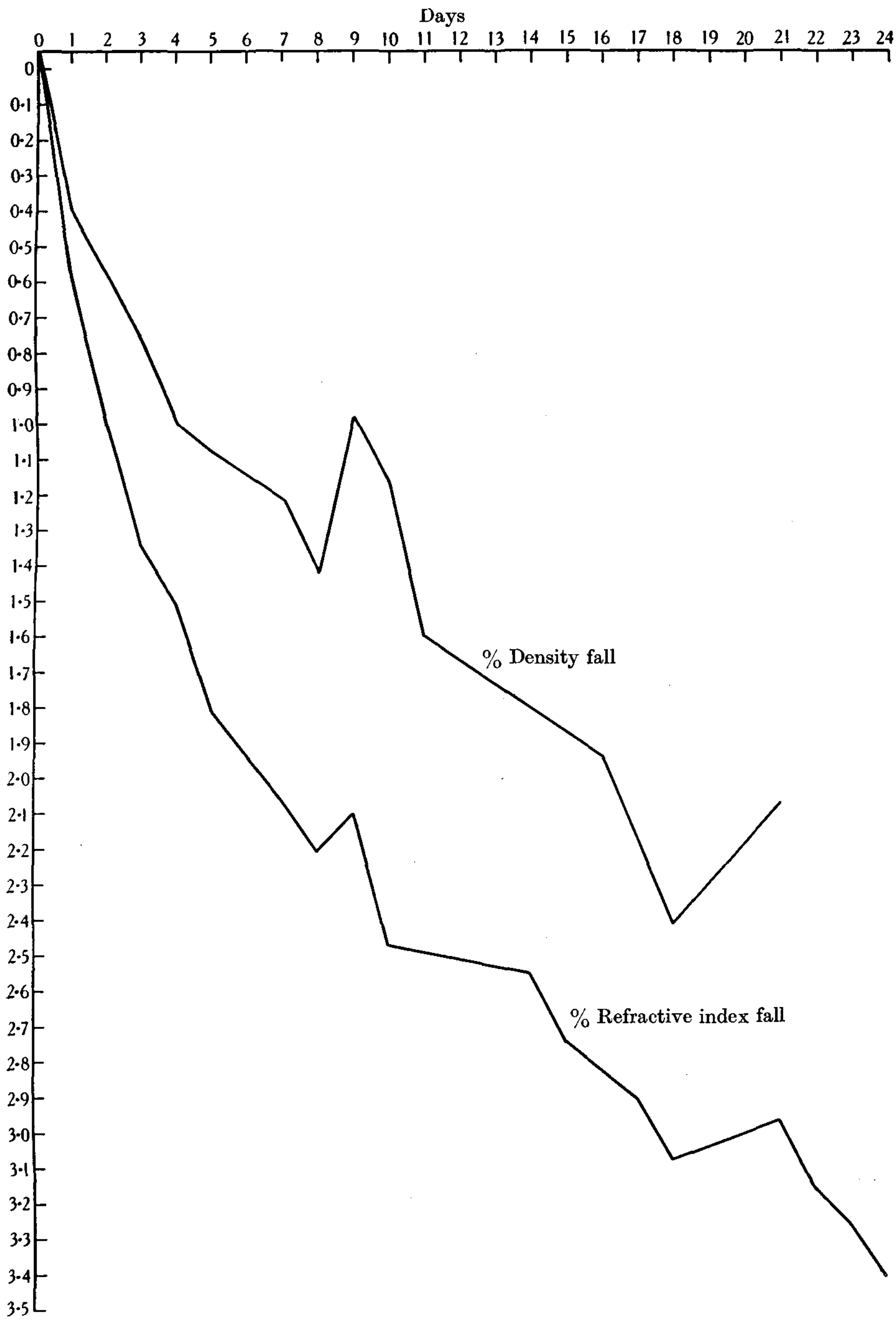

Graph 2. Oil 209 recovered from a series of animals.

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varying periods of time, as the acid treatment becomes more severe the index fall tends gradually to approximate to the density fall. The index-density fall ratio thus increases, but even when shaken with 35 per cent acid it does not reach the lowest figures given by the animal, although the absolute fall of both index and density were far greater in the former case. Figures more nearly comparable to those given by the animal were obtained by extracting the oil with different quantities of alcohol, and by successive extractions of a single lot of oil with a given volume of alcohol. But the records of these experiments, shown in Table III, indicate again a relatively small index fall compared with

Table III. *Residual oils after extraction with absolute alcohol*

Oil 209	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Original oil	0·9203	1·5125	0·5569	—	—	—	—
Treated with 100 % alcohol	0·9171	1·5104	0·5565	0·0032	0·0021	0·0004	0·656
,, 150 ,,	0·9160	1·5093	0·5560	0·0043	0·0032	0·0009	0·744
,, 200 ,,	0·9147	1·5087	0·5561	0·0056	0·0038	0·0008	0·679
,, 250 ,,	0·9132	1·5074	0·5556	0·0071	0·0051	0·0013	0·718
,, 300 ,,	0·9121	1·5066	0·5554	0·0082	0·0059	0·0015	0·720
,, 350 ,,	0·9117	1·5060	0·5550	0·0086	0·0065	0·0019	0·765
Successive treatments with 100 % alcohol							
1	0·9171	1·5104	0·5565	0·0032	0·0021	0·0004	0·656
2	0·9141	1·5081	0·5558	0·0062	0·0044	0·0011	0·710
3	0·9112	1·5059	0·5552	0·0091	0·0066	0·0017	0·725
4	0·9078	1·5032	0·5543	0·0125	0·0091	0·0026	0·728
5	0·9049	1·5010	0·5537	0·0154	0·0115	0·0032	0·747
6	0·9010	1·4982	0·5529	0·0193	0·0143	0·0040	0·741

the density fall, the index-density fall ratio of the oil recovered from the animal being only in one instance (18th day) lower than those given by the larger amounts of alcohol. Again we note that, as with acid treatment, there is a tendency for the index-density fall ratio to increase as the severity of the treatment increases. As a matter of fact it surpassed that of the acid-treated oils in most cases although the absolute falls of both index and density were less.

Oxygenation of oils has been attempted by several methods, including simple oxygenation at various temperatures, and oxidation with H_2O_2 with and without $CuSO_4$ and with and without NaOH. Some sample figures obtained are given in Table IV, a glance at which shows that, on the whole, there is a tendency towards a rise in density and index, especially the former, with

Table IV. *Alterations in density and index following oxidation of mineral oils*

Oil and treatments	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall
Original oil 8 (2)	0·8933	1·5034	0·5635	—	—	—
Oxygenated at 100°	0·8949	1·5040	0·5632	+0·0016	+0·0006	0·0003
,, and ultraviolet light	0·8987	1·5040	0·5608	+0·0054	+0·0006	0·0027
Original oil 47 (2)	0·9209	1·5132	0·5573	—	—	—
Oxygenated at 120°	0·9236	1·5145	0·5571	+0·0027	+0·0013	0·0002
,, 130°	0·9247	1·5146	0·5565	+0·0038	+0·0014	0·0008
,, 160°	0·9274	1·5145	0·5548	+0·0065	+0·0013	0·0025
Original oil 55	0·8935	1·5039	0·5640	—	—	—
Oxidized H_2O_2	0·8967	1·5040	0·5626	+0·0032	+0·0001	0·0014
,, H_2O_2, NaOH	0·8942	1·5037	0·5633	+0·0007	0·0002	0·0007
,, H_2O_2, $CuSO_4$	0·8941	1·5045	0·5643	+0·0006	+0·0006	+0·0003
,, H_2O_2, $CuSO_4$, NaOH	0·8943	1·5048	0·5645	+0·0008	+0·0009	+0·0005

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consequently a slight fall in refractivity. The figures thus bear not the slightest resemblance to those obtained with oils recovered from the animal body. Some further catalytic, electrolytic and ultraviolet light oxidations are now being carried out as the specimens available were manufactured several years ago and we are in some doubt as to the authenticity of the figures for the control oils.

Perhaps the most interesting procedure we have adopted in connexion with these experiments is that of reduction of oils by the action of sodium in boiling amyl alcohol. The results of two tests with shale oil were:

	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Original oil	0·8935	1·5039	0·5640	—	—	—	—
2 g. sodium to 5 c.c. oil	0·8926	1·4976	0·5575	0·0009	0·0063	0·0065	7·000
1 g. sodium to 5 c.c. oil	0·8929	1·4990	0·5589	0·0006	0·0049	0·0051	8·167

Here the index-density fall ratio is a remarkable one, being in one case as high as 8 to 1, with the refractivity fall in both instances higher than the index fall. The exaggerated figures obtained appear, however, to depend mostly on the formation of acidic compounds, removal of which modifies our figures considerably. A series of treatments of an oil with successive portions of sodium in amyl alcohol, followed by removal of the acidic compounds and controls treated with sodium amylate, gave the readings shown in Table V.

Table V. *Index and density fall after successive treatments of oil with sodium in amyl alcohol*

Oil 55	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Original oil	0·8935	1·5039	0·5640	—	—	—	—
Sodium: 1 part	0·8955	1·5040	0·5628	+0·0020	+0·0001	0·0012	—
2 parts	0·8954	1·5035	0·5623	+0·0019	0·0004	0·0017	—
3 ,,	0·8936	1·5017	0·5614	+0·0001	0·0022	0·0026	—
4 ,,	0·8924	1·5002	0·5605	0·0011	0·0037	0·0035	3·364
5 ,,	0·8919	1·4993	0·5598	0·0016	0·0046	0·0042	2·875
6 ,,	0·8914	1·4986	0·5593	0·0021	0·0053	0·0047	2·524
7 ,,	0·8909	1·4982	0·5592	0·0026	0·0057	0·0048	2·192
8 ,,	0·8914	1·4982	0·5589	0·0021	0·0057	0·0051	2·714
9 ,,	0·8913	1·4988	0·5596	0·0022	0·0051	0·0044	2·318
10 ,,	0·8910	1·4972	0·5580	0·0025	0·0067	0·0060	2·680
Control 1	0·8952	1·5045	0·5636	+0·0017	+0·0006	0·0004	—
,, 2	0·8949	1·5044	0·5636	+0·0014	+0·0005	0·0004	—
,, 3	0·8969	1·5040	0·5619	+0·0034	+0·0001	0·0021	—

Control 1 is the original oil simply treated with sodium amylate, the figures not being very different from those found when the oil was treated with the sodium in boiling amyl alcohol.

Control 2 is the original oil treated with five successive lots of sodium amylate, the figures obtained being very similar to those of Control 1, but very different from those of the oil treated with five parts of sodium in boiling amyl alcohol.

Control 3 was similar to Control 2 with the exception that the oil was acidified previous to treatment. The reason for the exceptional increase in density is not very obvious to us.

THE INJECTION OF CHEMICALLY TREATED OILS INTO ANIMALS

The next step was to test the animal reaction to our specially treated oils, and compare it with the reaction to the injection of control oils, the latter being straight distillates from the crude oil with sufficient acid and clay treatment to render them presentable to the market. In Table VI are shown the results

Table VI. *Index and density fall after extraction with alcohol of oils recovered from animals*

Oil 209, 100 vols.	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Alcohol: 1 treatment	0·9171	1·5104	0·5565	0·0032	0·0021	0·0004	0·656
1 day	0·9142	1·5076	0·5552	0·0029	0·0028	0·0013	0·966
2 days	0·9121	1·5058	0·5545	0·0050	0·0046	0·0020	0·920
3 ,,	0·9093	1·5043	0·5546	0·0078	0·0061	0·0019	0·782
4 ,,	0·9106	1·5037	0·5532	0·0065	0·0067	0·0033	1·031
5 ,,	0·9073	1·5022	0·5535	0·0098	0·0082	0·0030	0·837
7 ,,	0·9057	1·5013	0·5535	0·0114	0·0091	0·0030	0·798
8 ,,	0·9034	1·4992	0·5526	0·0137	0·0112	0·0039	0·818
9 ,,	0·9051	1·4998	0·5522	0·0120	0·0106	0·0043	0·883
10 ,,	0·9051	1·4990	0·5513	0·0120	0·0114	0·0052	0·950
11 ,,	0·9087	1·5032	0·5538	0·0084	0·0072	0·0027	0·857
Alcohol: 6 successive treatments	0·9010	1·4982	0·5529	0·0193	0·0143	0·0041	0·741
1 day	0·9009	1·4975	0·5522	0·0001	0·0007	0·0007	7·000
2 days	0·9003	1·4968	0·5518	0·0007	0·0014	0·0011	2·000
3 ,,	0·8995	1·4962	0·5516	0·0015	0·0020	0·0013	1·333
4 ,,	0·9005	1·4972	0·5521	0·0005	0·0010	0·0008	2·000
5 ,,	0·8977	1·4952	0·5516	0·0033	0·0030	0·0013	0·909
7 ,,	0·8973	1·4947	0·5513	0·0037	0·0035	0·0016	0·946
8 ,,	0·8964	1·4945	0·5517	0·0046	0·0037	0·0012	0·804
9 ,,	0·8964	1·4942	0·5513	0·0046	0·0040	0·0016	0·870
10 ,,	0·8965	1·4938	0·5508	0·0045	0·0044	0·0021	0·978
11 ,,	0·8954	1·4936	0·5513	0·0056	0·0046	0·0016	0·821

obtained by the injection of oil 209, once and six times extracted with 100 volumes of ethyl alcohol.

The residual oils left over after removal of some of the toxic ingredients by the alcohol are of necessity less vigorously attacked by the animal organism than the control oil (Table I), the small fall in index and density being especially noticeable in the residue left after six extractions. It will also be noticed that with the sixth residue the index fall for the first 4 days of the experiments tends to be higher than the density fall, this being partly due to the low toxicity of this residue and the fact that the treatment *in vitro* resulted in a relatively small index fall compared with that of the density fall, the ratio being only 0·741 : 1.

The injection of acid-treated oils was not resorted to, as the animals would not well tolerate the oils treated by us with sulphuric acid, even although the oils were repeatedly washed. Oils oxygenated at a temperature of much above 100° C. were also not well tolerated, but oxidation with H_2O_2 did not seem to increase the toxicity, and the injection of oils so treated was undertaken. The results of two experiments together with the control are shown in Table VII.

As with the alcohol residues, the chief thing to note is the levelling action of the animal organism. The relatively high density of the oxidized oil is rapidly lowered by the animal, until in about a week's time the physical

Table VII. *Index and density fall in oil recovered from series of injected mice*

Oil 55	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Original oil	0·8935	1·5039	0·5640	—	—	—	—
Animal residue: 1 day	0·8920	1·5020	0·5628	0·0015	0·0019	0·0012	1·266
2 days	0·8900	1·5002	0·5620	0·0035	0·0037	0·0020	1·057
3 ,,	0·8881	1·4995	0·5624	0·0054	0·0044	0·0016	0·815
4 ,,	0·8864	1·4975	0·5613	0·0071	0·0064	0·0027	0·901
5 ,,	0·8875	1·4972	0·5602	0·0060	0·0067	0·0038	1·117
7 ,,	0·8860	1·4973	0·5613	0·0075	0·0066	0·0027	0·880
8 ,,	0·8836	1·4953	0·5605	0·0099	0·0086	0·0035	0·869
Control treated H_2O_2	0·8967	1·5040	0·5626	+0·0032	+0·0001	0·0014	—
Animal residue: 1 day	0·8920	1·5022	0·5630	0·0047	0·0018	+0·0004	0·383
2 days	0·8905	1·5005	0·5620	0·0062	0·0035	0·0006	0·565
3 ,,	0·8865	1·4987	0·5625	0·0102	0·0053	0·0001	0·520
4 ,,	—	1·4988	—	—	0·0052	—	—
5 ,,	0·8871	1·4978	0·5612	0·0096	0·0062	0·0014	0·646
7 ,,	0·8863	1·4974	0·5612	0·0104	0·0066	0·0014	0·635
8 ,,	0·8846	1·4961	0·5608	0·0121	0·0079	0·0018	0·653
9 ,,	0·8841	1·4960	0·5610	0·0126	0·0080	0·0016	0·635
10 ,,	—	1·4957	—	—	0·0083	—	—
Control treated H_2O_2, NaOH	0·8942	1·5037	0·5633	+0·0007	0·0002	0·0007	—
Animal residue: 1 day	0·8932	1·5024	0·5625	0·0010	0·0013	0·0008	1·300
2 days	—	1·5007	—	—	0·0030	—	—
3 ,,	0·8879	1·4991	0·5621	0·0063	0·0046	0·0012	0·730
4 ,,	0·8880	1·4989	0·5618	0·0062	0·0048	0·0015	0·774
5 ,,	0·8880	1·4984	0·5613	0·0062	0·0053	0·0020	0·855
7 ,,	—	1·4972	—	—	—	—	—
8 ,,	0·8842	1·4958	0·5607	0·0100	0·0079	0·0026	0·790
9 ,,	0·8824	1·4950	0·5610	0·0118	0·0087	0·0023	0·737
10 ,,	—	1·4947	—	—	0·0090	—	—

characteristics of the oxidized oils are brought almost to the level of that of the control oil, the index-density fall ratio, of course, still remaining well below that of the control oil.

The results of the injection of shale oil 55 and the oil reduced by the action of sodium in amyl alcohol are shown in Table VIII. The reduction of the oil having so markedly affected the refractive index, with only an accompanying slight change in the density, the levelling action of the animal organism when

Table VIII. *Index and density fall after injection of oil treated with sodium in boiling amyl alcohol*

Oil 55	Density	Refractive index	Refractivity	Density fall	Refractive index fall	Refractivity fall	Refractive index fall / Density fall
Control B	0·8935	1·5039	0·5640	—	—	—	—
1 day	0·8925	1·5021	0·5626	0·0010	0·0018	0·0014	1·800
2 days	0·8921	1·5009	0·5615	0·0014	0·0030	0·0025	2·144
3 ,,	0·8895	1·4997	0·5618	0·0040	0·0042	0·0022	1·050
4 ,,	0·8894	1·4987	0·5607	0·0041	0·0052	0·0033	1·268
5 ,,	0·8882	1·4973	0·5599	0·0053	0·0066	0·0041	1·245
7 ,,	0·8862	1·4960	0·5597	0·0073	0·0079	0·0043	1·082
8 ,,	0·8830	1·4946	0·5601	0·0105	0·0093	0·0039	0·886
9 ,,	0·8858	1·4957	0·5596	0·0077	0·0082	0·0044	1·065
10 ,,	0·8847	1·4948	0·5593	0·0088	0·0091	0·0047	1·034
Treated oil	0·8926	1·4976	0·5575	0·0009	0·0063	0·0065	7·000
1 day	0·8891	1·4968	0·5588	0·0035	0·0008	+0·0013	0·229
2 days	0·8875	1·4955	0·5583	0·0051	0·0021	+0·0008	0·412
3 ,,	0·8861	1·4948	0·5584	0·0065	0·0028	+0·0009	0·431
4 ,,	0·8860	1·4945	0·5581	0·0066	0·0031	+0·0006	0·470
5 ,,	0·8843	1·4933	0·5578	0·0083	0·0043	+0·0003	0·518

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the reduced oil is injected is very pronounced. Again, the density fall of the reduced oil after injection being relatively greater than the index fall, there is a rise in the refractivity, a state of affairs which, when met with elsewhere, is usually considered to be due to animal variation. It will be noted that there is a tendency for the refractivity rise from day to day gradually to become less. When the control oil was diluted with 50 per cent of an inert mineral oil, and injected into animals, the results were very similar to those given by the undiluted oil, with the exception, of course, that both index and density fall were less in degree in the former case. When, however, the reduced oil was similarly diluted, the animal results were not nearly so clearly cut as with the undiluted oil. There was also nothing very remarkable in the results given by the reduced oils from which the acidic compounds were subsequently removed, these results conforming to those found in previous experiments.

The injection of miscellaneous oils

Among a variety of other experiments related to our subject were those undertaken with a series of synthetic oils kindly provided by Prof. Nash. These oils were prepared from ethylene by the use of aluminium chloride, Nos. 330, 331 and 332 at a temperature of 10–15° C. and Nos. 333 and 334 at a temperature of 300° C. The properties of these oils determined by us at 25° C. were:

Oil no.	Density	Refractive index	Refractivity
330	0·8834	1·4819	0·5455
331	0·8863	1·4828	0·5447
332	0·9015	1·4900	0·5435
333	0·8690	1·4767	0·5486
334	0·8816	1·4857	0·5509

Unfortunately quantities sufficient for viscosity measurements were not available. Owing to the fact that the refractivities for mineral oils were low, it was not anticipated that they would call forth much reaction on the part of the animal, but it was soon evident that they were badly tolerated, most of the animals dying on injection of the pure oils. Dilution with an inert mineral oil resulted in unexpected findings in the oils recovered from the animals, although even the diluted oils were not well tolerated. The indices were not appreciably altered but the density was in pure oil 334 raised 0·0060 and in the 50 per cent dilution of oils 330 and 331 raised 0·0052 and 0·0033 respectively, the final refractivities becoming in consequence very low.

In further experiments the oil recovered from several hundred mice 7 days after the injection of oil 102 was pooled in two different portions, the properties of which were:

Oil	Density fall	Index fall	Refractivity fall	$\frac{\text{Index fall}}{\text{Density fall}}$
Specimen 1	144	133	60	0·925
Specimen 2	133	128	61	0·962

Oils recovered from a series of animals injected with specimen 2 and a control oil gave the following figures for refractive index fall, those relating to density not being for the moment available:

	Refractive index fall	
Day	Control	Specimen 2
1	0·0047	0·0009
2	0·0059	0·0013
3	0·0109	0·0018
4	0·0116	0·0021
5	0·0123	—
7	0·0136	0·0030
8	0·0162	0·0032
9	0·0152	0·0037
10	0·0161	0·0032
11	0·0165	—

This experiment was designed to find out whether an animal which had 7 days during which to detoxicate an oil would continue to detoxicate to a degree similar to that of a fresh animal. The animal which had been in contact with the oil for 7 days might have become enfeebled by its efforts to detoxicate or it might have become more sensitive by virtue of the development of tolerance or immunity. On the other hand, the quantity of oil remaining free in the peritoneal cavity on the 7th day should be considerably less than that injected, so that from this point of view the 8th day control animal should have a lighter task to perform than the 1st day animal injected with specimen 2. While from the above figures it appears that this may indeed be the case, we do not consider that our results, on the whole, are clear enough to warrant definite conclusions. Other experiments have not helped us much in this respect (compare those given by oil 209 in Table I). It also has to be remembered that although the amount of oil free in the peritoneal cavity gradually diminishes with time, we have no evidence that any of the oil is excreted by the animal, it probably being removed only to other parts of the body or trapped locally. We know, of course, that at least some of the injected oil is trapped by the proliferating endothelial cells lining the peritoneal cavity, the amount of oil trapped in a given time increasing with increasing unsaturation and viscosity. The trapped oil when recovered from the tissues has a refractive index fall approximately similar to that of the free oil, in view of which it does not appear that there is a trapping of selected constituents of the injected oil.

The possibility of the reaction in the animal being a surface one was investigated by utilizing emulsions of mineral oil made up with sodium oleate, gum, gelatine, etc. For various reasons the oleat emulsion is the sole one so far used for injection, and from experiments with this emulsion there were indications that the oil was changed a little more rapidly than is ordinarily the case with pure oil and that there is a slightly lowered index-density fall ratio.

The difficulty of obtaining chemically pure carcinogenic hydrocarbons in solution in sufficient quantity to alter materially the refractive index of solvents suitable for injection into animals has obliged us to turn our attention to hydrocarbons of lower molecular weight without carcinogenic properties. Among the substances tested were acenaphthene, phenanthrene and tetrahydrophenanthrene, from 2·5 to 5 per cent being dissolved in slab oil or squalene. When the solutions were injected into the peritoneal cavity of animals and subsequently recovered in the ordinary manner, very little evidence of the presence of the hydrocarbons was found even as soon as 24 hours after the injection. The results were, however, not parallel with the two solvents with all three substances, nor were they parallel as regards index fall and density fall. There are several discrepancies in the results of this group of experiments which require further investigation before discussion of them is worth while.

An attempt is at present being made to gain a further insight into the animal mechanism, by separating toxic oils into fractions by other than purely chemical means, and injecting the fractions into animals. The method of separation consists in distilling the oil at very low pressures (of the order of 0·01–0·001 mm. mercury) over very narrow ranges of temperature in a specially designed still along the lines indicated by the work of Hickman and his collaborators. These experiments are at present not far advanced, but already they show promise of giving some very interesting results.

The part played by viscosity

It has been remarked previously that, other things being equal, the lower the viscosity of an injected oil, the sharper the animal response. This, perhaps, is best exemplified by the examination of a series of cuts from the distillation of a single crude oil. In Table IX are shown the results obtained with

Table IX. *Index and density fall in distillation cuts from Peruvian naphthenic base crude oil*

Cut no.	Oil no.	Density	Refractive index	Refractivity	Kinematic viscosity	Density fall	Refractive index fall	Refractivity fall
1	341	0·8851	1·4856	0·5486	10·16	0·0119	0·0088	0·0026
2	342	0·8889	1·4881	0·5491	18·88	0·0103	0·0075	0·0021
3	343	0·8934	1·4910	0·5496	27·57	0·0090	0·0067	0·0020
4	344	0·8967	1·4930	0·5498	36·02	0·0078	0·0058	0·0017
5	345	0·8991	1·4944	0·5499	52·49	0·0063	0·0048	0·0015
6	346	0·9020	1·4962	0·5501	77·36	0·0039	0·0032	0·0012
7	347	0·9044	1·4973	0·5499	116·2	0·0014	0·0024	0·0018
8	348	0·9096	1·5003	0·5500	181·4	0·0022	0·0023	0·0012
9	349	0·9148	1·5032	0·5501	275·9	0·0023	0·0025	0·0026
10	350	0·9192	1·5053	0·5497	497·4	0·0023	0·0020	0·0008
11	351	0·9213	1·5067	0·5500	826·2	0·0012	0·0010	0·0004
12	352	0·9232	1·5081	0·5504	1131·0	0·0015	0·0009	0·0001
13	353	0·9239	1·5089	0·5508	1454·0	0·0004	0·0007	0·0005
14	354	0·9270	1·5114	0·5517	2117·0	0·0008	0·0001	+0·0003
15	355	0·9287	1·5132	0·5526	2954·0	0·0003	0·0005	0·0004
16	356	0·9315	1·5156	0·5535	4458·0	+0·0002	0·0011	0·0013
17	357	0·9361	1·5188	0·5542	7951·0	—	0·0005	—

seventeen cuts from a Peruvian naphthenic base crude oil kindly provided by Messrs Lobitos. Here we have, as usual, a steady rise in density, index and viscosity, the rise in refractivity being slight except towards the end of the distillation. The small irregularities in some of the earlier figures are partly due to the refining processes to which the cuts were subjected having been varied slightly as the distillation temperature increased.

As regards the reaction of the animal to the injected oils, it is evident that the gradual lack of response of the animal as the viscosity, index and density rise is to be associated with viscosity rather than with index and density. We have learned from previous experiments that with oils of similar viscosity the animal usually attacks those with high indices and densities more vigorously than it does those in which these characteristics are low.

The chemical treatments of the oils by extraction with alcohol opens up some interesting points in relation to carcinogenic potency and viscosity. Successive extraction of oils with ethyl alcohol does not give parallel results as far as viscosities are concerned, although it does seem to do so as regards carcinogenic potency. In Table X are given the viscosities of the oils obtained

Table X. *Kinematic viscosities of alcohol extracts and residues of three mineral oils*

	Oil 55, 51·86		Oil 102, 34·82		Oil 209, 72·03	
No.	Extract	Residue	Extract	Residue	Extract	Residue
1	101·6	48·85	57·24	—	57·88	81·62
2	79·30	44·92	41·79	33·17	54·85	81·90
3	66·49	47·24	38·03	32·96	55·69	82·80
4	62·79	46·57	36·44	33·18	57·13	87·24
5	58·59	46·09	35·90	33·01	61·53	94·16
6	58·48	46·56	34·31	33·16	64·78	96·89

The difficulty of removing the last traces of alcohol rendered these viscosity determinations less reliable than those carried out on ordinary oils.

by the successive extraction with alcohol of shale oil 55, Persian oil 102 and Mexican-Venezuelan blend oil 209. It will be noted that while with oils 55 and 102 the first extract is of a high viscosity, the viscosity of subsequent extracts and the residues gradually becomes less. In the case of oil 209 the results are reversed, the first extract being of a lower viscosity than the original oil, with the subsequent extracts and residue gradually rising in viscosity. Now in all three oils, as in many others we have extracted with alcohol, the extracts are found invariably to contain the high density and high index ingredients, while at the same time they have proved to be definitely more carcinogenic than the residues in every instance tested, whether by painting the skin or by intraperitoneal injection. Consequently, as the viscosity is to a considerable extent a reflection of the boiling range one can deduce that the active constituents of, for instance, oil 209, have a lower boiling range than the average constituents, while in the case of oils 55 and 102 the conditions are reversed.

These experiments verify our original contention that the viscosity of an oil *per se* plays but little part in influencing the extent of the animal reaction, the latter being dependent upon the degree of unsaturation or dehydrogenation and the molecular weight of the constituents of the oil.

We are still not in a position to say by what means the animal is enabled to alter the physical characteristics of mineral oils. Dr Bottomley is at present examining this aspect of the question from the theoretical and mathematical side, and meanwhile it appears that all one can say is that the process is certainly not solely one of selective removal of the toxic ingredients of the oil from the peritoneal cavity or local trapping of them, but that there is a preliminary chemical change which in all probability is followed by removal of the changed products.

The number of determinations made in connexion with this work has been very large and we wish to acknowledge here the help we have received from our assistants, especially Mr F. Dixon and Mr L. Norburne for the density determinations, and Mr J. G. Cox and Mr R. A. Combes for the index and viscosity determinations.

Summary and conclusions

1. The fall in the refractive index of mineral oils recovered after injection into the peritoneal cavity of mice is usually accompanied by a fall in the density proportional to the fall in the index.

2. With straight distillates from the crude oil the ratio of index fall to density fall is on an average about 0·85 to 1. Wide deviations from these figures were rarely found except when dealing with oils of low toxicity. The ratio tends to be above the mean when the actual index fall is low and consequently below the mean when the index fall is high.

3. The refractivity of the original oil, the index fall of the recovered oil and the density fall of the recovered oil give information as to the carcinogenic potency for mice of the original oil of about equal value, but the two last-mentioned tests are far superior to the first for assessing the probable dermatitic potency of the oil.

4. Other things being equal, the index and density fall are closely dependent upon the index, density and viscosity of the original oil.

5. The index and density fall gradually become larger as the experiment progresses, but within each 24-hour interval the fall gradually becomes less. The ratio of index fall to density fall remains fairly constant for each individual oil during the course of the experiment.

6. Titration of mineral oils *in vitro* with sulphuric acid and alcohol provides residual oils of lower index and density than the original oil, but the ratio of the fall in index to the fall in density is low, especially when the treatments are not severe.

7. Oxidation of mineral oils tends to raise the density without affecting the index to an appreciable extent. On the other hand reduction tends to lower the index without affecting the density to anything like the same extent.

8. In general terms the animal reacts upon the chemically treated oils in an additive manner, so that if by the chemical treatment we have the ratio of index fall to density fall low, the oil recovered from the animal will have the ratio high, and vice versa. The end product of the severe treatment of a mineral oil with acid and clay is a white oil which for practical purposes may be

considered to be fully saturated. The end product of an oil recovered from the animal is probably similar in nature, although the process involved in arriving at this end product is presumably different chemically and physically.

9. Synthetic oils recovered from the animal after injection showed a marked rise in density but no corresponding rise in index.

10. The animal reaction to an injected oil was not materially accelerated by emulsification of the oil.

11. Some chemically pure non-carcinogenic compounds were very rapidly changed or eliminated from the peritoneal cavity.

12. All alcohol extracts of mineral oils tested showed an increase in index, density and refractivity, but not always in viscosity compared with the original oil, the residues, of course, having characteristics in an opposite direction. The latter were in every instant less toxic for the animal than the original oil, whether applied to the skin or injected.

13. A mineral oil recovered from animals 7 days after injection, when reinjected into a second series of animals, was altered as regards its physical characters in a normal manner. Thus whether allowed to remain in the peritoneal cavity of an animal of the first series or recovered and reinjected into an animal of the second series, the ultimate index and density fall in a given time were approximately similar.

14. The free oil in the peritoneal cavity becomes, from day to day, less. This is at least partly due to local trapping by the proliferating endothelium. The fall in index of the trapped oil is approximately similar to that of the free oil.

15. The alteration of the physical characteristics of mineral oils by the animal is probably brought about by a chemical reaction followed by removal of the changed product.

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