CONCENTRATION OF THE CARCINOGENIC MATERIAL IN A VENEZUELAN SPINDLE OIL BY SIMULTANEOUS MOLECULAR DISTILLATION AND CHROMATOGRAPHIC ADSORPTION

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EXPERIMENTS WITH VENEZUELAN SPINDLE OIL

The Venezuelan spindle oil, our No. 46(2), was separated into nine distillates and a residue by simultaneous molecular distillation. Altogether about 13·8 of the oil were distilled. In Table 1 are given some of the physical constants of the control oil and its simultaneous molecular distillates together with the percentage volume of each. The abbreviations used are: RI = refractive index; KV = kinematic viscosity; RC = refractivity; RIF = fall in refractive index of oil recovered from the peritoneal cavity of mice after 7 days; K under RIF indicates that the injected mouse died before the seventh day; D = density; Vol. = percentage volume of total oil. All physical constants taken as usual at 25° C.

Table 1

Agent	RI	KV	RC	RIF	D	Vol.
46(2)	1·5137	60·7	5570	K, 2nd day	0·9223	100
Dist. 1	1·5135	19·4	5586	K, 2nd day	0·9192	5·44
,, 2	1·5133	18·8	5584	K, next day	0·9193	7·97
,, 3	1·5147	22·6	5581	K, 2nd day	0·9223	9·42
,, 4	1·5145	26·8	5577	K, 3rd day	0·9225	9·06
,, 5	1·5152	33·3	5577	K, 2nd day	0·9237	7·97
,, 6	1·5147	52·7	5578	167	0·9227	8·70
,, 7	1·5138	81·8	5574	114	0·9218	15·94
,, 8	1·5120	142·9	5569	88	0·9194	16·67
,, 9	1·5117	258·4	5557	57	0·9208	14·50
Residue 10	1·5200	935·3	5596	K, 2nd day	0·9292	4·35

Owing to its deep colour the index of the residue could only be very approximately determined.

Scrutiny of the table reveals that with the exception of the residue there is not much difference in the refractive indices or densities and therefore refractivities of the various distillates. There is, however, a very large difference in viscosities, and for this reason we expected the middle distillates to show the greatest carcinogenic activity and the early distillates to show relatively more dermatitic activity. As will be seen later this was confirmed by the skin-painting results. Consideration of the table also reveals that only mice injected with distillates 6–9 survived until the seventh day.

Two series of skin-painting experiments have now been completed with these distillates and the control oil. The mice were painted between the shoulder blades five times weekly for the first few weeks and then subsequently bi-weekly until 45 weeks, when all surviving mice were killed. The first series of experiments comprising twenty-five mice in each experiment were performed with distillates 2, 4, 6, 8 10, and the control oil 46(2). The death-rate was high, especially among the mice treated with the early distillates, where ulcerative dermatitis was produced in many of the animals after a few weeks' painting. Although no tumours were produced in the mice painted with distillates 2 and 4, it was obvious from microscopic examination of many skins that tumours would have been produced in some of these animals if they had lived longer. Even though the death-rate was relatively low among the mice painted with distillate 10, only a few benign tumours were produced and only an occasional mouse was observed with a skin ulcer in this experiment.

The second series of experiments were started some months later when a different batch of mice were utilized. For these reasons a second experiment was performed as control to distillates 1, 3, 5, 7, and 9. Thirty-five mice were utilized in each of these experiments, but again the death-rate was high except among the mice painted with nos. 1 and 9. A single tumour was produced in the experiments with nos. 1 and 3, which fact confirmed that a certain amount of carcinogenic material was present in these early distillates. Although many of the mice painted with distillate 1 exhibited ulcerative dermatitis, this material, in our opinion, was probably not quite so active in this respect as distillates 3 and 4. Nine tumours arose in distillate 9, but the tumours arose later on an average than did those which appeared in the control mice. There was, moreover, less tendency for the mice treated with distillate 9 to develop skin ulcers than in the controls. Thus this distillate, although apparently appreciably more carcinogenic than no. 10, was probably somewhat less carcinogenic and certainly less dermatitic than the control.

In the tables set out below, which give particulars of our results with the two series of experiments, abbreviations are as follows: No. = number of mice utilized for each experiment; No. 1 = number of survivors at 20, 35, and 45 weeks respectively; T = total tumours at these periods;

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C = addition cumulative frequency, and by that is meant the percentage number of mice which it is assumed would have borne tumours at a particular period had all the mice survived until then. To obtain this number tumours are allocated each week to mice which die tumourless in the proportion in which they arise in living mice (Twort & Twort, 1933); M = total number of mice throughout the experiments which were considered to bear malignant tumours; P = potency which is derived from the mean of our method 1 and a slight modification of our method 2 (Twort & Twort, 1933); D = duration of experiment in weeks.

Table 2

		No. 1			T			C					
Agent	No.	20	35	45	20	35	45	20	35	45	M	P	D
46(2)	25	7	3	1	2	7	7	28	100	—	2	78	45
Dist. 2	25	5	2	0	0	0	—	0	0	0	0	0	43
Dist. 4	25	4	2	1	0	0	0	0	0	0	0	0	45
Dist. 6	25	10	1	0	8	9	—	55	100	—	4	389	30
Dist. 8	25	6	0	0	4	5	—	43	100	—	0	90	29
Dist. 10	25	19	9	4	2	3	4	10	16	33	0	9	45

Table 3

		No. 1			T			C					
Agent	No.	20	35	45	20	35	45	20	35	45	M	P	D
46(2)	35	12	4	2	2	5	5	16	60	60	1	27	45
Dist. 1	35	19	7	2	1	1	1	5	5	5	0	5	45
Dist. 3	35	7	2	0	0	1	1	0	33	33	0	5	41
Dist. 5	35	10	2	1	4	5	5	24	37	100	1	35	45
Dist. 7	35	10	0	0	5	7	—	28	100	—	1	109	32
Dist. 9	35	17	9	2	3	6	9	14	55	100	3	30	45

We know from experience that in experiments where the death-rates are high, more reliable information can sometimes be obtained by considering data obtained during the relatively early stages of such experiments. If therefore we consider in the tables the number of survivors, the total tumours and the addition cumulative frequency at 20 weeks we shall probably have a better guide as to the relative activity of the various distillates and the control oil. Consideration of the addition cumulative frequency, which is probably the best guide, at 20 weeks indicates that distillates 6 and 8 are perceptibly more carcinogenic than the control, the figures being 55, 43, and 28, respectively. Distillate 10, which gives only a frequency of 10, is, however, apparently much less carcinogenic. The potency figures for distillates 6, 8, 10 and control are in the same order, but the relative potency of 6 (389) and 8 (90) compared to the control (78) is probably too high and too low respectively.

Particulars as regards the second series of experiments indicate the importance of the second control. The addition cumulative frequency figures at 20 weeks for this control is only 16, whereas distillate 7, which is apparently the most carcinogenic of this series, gives a figure of 28, and this it will be remembered is the same as that given by the control to the first series. It is probable that both distillates 5 and 7 are more carcinogenic than the control and as suggested before distillate 9 somewhat less active. We are of this opinion as regards distillate 9, even though the potency figures, the number of tumours diagnosed as malignant, and the cumulative frequency at 45 weeks are in advance of those given by the control. The relative healthier condition of the skin in many of the mice comprising this experiment, presumably due to the presence of less dermatitic material in distillate 9, would account for more of the tumours taking on a malignant character in this experiment compared to the control. As regards the other distillates we may note that the cumulative frequency figures at 20 weeks correlate very well with the potency figures. The figures given for distillates 1 and 3 are of value only from a negative point of view, as only one tumour was produced in each of these experiments.

Examination of the tables shows that the production of tumours classified as malignant was small, but this of course is understandable when one considers the death-rate. It is noteworthy, however, that such tumours were produced only in both control experiments and in those with distillates 5, 6, 7 and 9. We are not surprised that no malignant tumours were produced among the mice painted with distillate 8, as the experiment was relatively of such short duration.

To sum up, we can say that in spite of the relatively poor data available from these experiments we are confident that distillates 6 and 7 are almost certainly more carcinogenic than the control, and that 5 and 8, more especially the latter, are probably more carcinogenic. Distillate 9 is probably somewhat less carcinogenic and certainly less liable to cause ulcerative dermatitis. Distillates 1–4 are less carcinogenic, but 2–4 somewhat more liable to cause ulcers than the control. The residue no. 10 is apparently the least active as regards dermatitis but probably more active as regards the production of tumours than the early distillates, nos. 1–4.

CHROMATOGRAPHIC ADSORPTION

Particulars regarding the technique employed during the chromatographic adsorption of these and other oil distillates, including shale, will be published elsewhere.

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We might mention, however, that before utilizing this method we subjected distillate 6 to molecular redistillation in the hope of effecting some further concentration in this way. Meanwhile we subjected distillate 7 to chromatographic adsorption, and skin-painting experiments have been performed on a few mice with what we considered to be the two most active fractions from this distillate. With the exception of no. 1, with which an experiment is still in progress, we have not meanwhile tested other fractions as we had previously tested fractions corresponding in many respects to these which we had obtained from four molecular distillates of a shale oil. Numerous experiments on the skin with these chromatographic products of shale oil indicated that fraction 1 was inert or practically inert. This was shown not only from its effect on the skin but also from the fact that its refractive index showed no reduction after 7 days' sojourn in the peritoneal cavity of mice. The second fractions tested in the same way showed definite activity, but their action on the skin was mainly dermatitic whereas the third fractions reacted very like their original distillates. The residues left in the adsorption columns were usually less active on the skin, although they killed injected mice. An exception was a residue from which some crystalline material later separated, and this residue in its original form was rather more carcinogenic than its parent distillate. An experiment performed on a few mice with a 0·1 % solution in chloroform of some crystalline material obtained from one of these shale distillates produced only one papilloma among five mice. This crystalline material was usually associated with a certain amount of viscous oil having a very high refractive index, and we are at present testing this viscous material on the skin as a 1 % solution in chloroform.

In Table 4 below are given particulars of the refractive index, the kinematic viscosity, and the approximate quantity of the different chromatographic fractions obtained from 100 c.c. of distillate 7 of oil 46(2). Abbreviations are: RI = refractive index; KV = kinematic viscosity; and Q = quantity of the various fractions:

Table 4

	RI	KV	Q
Distillate 7	1·5137	77·3	100
Fraction 1	1·4752	41·7	10
„ 2	1·4968	60·0	20
„ 3	1·5195	75·7	65
„ 4	1·6340	Very viscous	2·5
„ 5	—	Solids	0·16 g.
Residue	1·5131	Very viscous	2·5

Since from fraction 4 a small amount of crystalline solid material afterwards separated and fraction 5 contained a certain amount of viscous oil, we added these two fractions together and made a rough separation by means of alcohol into a highly viscous fraction 4 and a fraction composed almost entirely of crystalline solids, fraction 5. It is the particulars of these modified fractions and not the originals which are given in Table 4, and it is also these modified fractions which we utilized for our skin-painting experiments.

Animal experiments

Three experiments with a 1 % solution in chloroform of no. 4, no. 5 and the control oil, distillate 7, have now been in progress for some 36 weeks. Five mice were utilized for each of these experiments, and unfortunately none of the mice painted with fraction 4 survived after 26 weeks. However, four of the mice from this experiment developed skin tumours, and the same number of animals painted with fraction 5 have so far skin tumours but one mouse still tumourless survives. On the other hand, none of the mice painted with the control oil has so far given tumours and there are still four survivors in this experiment. Two further experiments have now been completed, one with the control distillate 7 and the other with a mixture of all the chromatographic fractions from distillate 7 except nos. 4 and 5. Incidentally, the refractive index of this mixture was 1·5114 compared to the control which was 1·5137; an indication that the mixture was probably somewhat less active than the control. Twenty-five mice were utilized for each of these two experiments, and the oils were applied daily undiluted. The death-rates, as is usual when dealing with undiluted toxic oils, were very high, but sufficient data were obtained to prove that, as expected from a consideration of the relative refractive indices, the mixture was not much less active than the control. Three mice only developed tumours in the control experiment, and all of the mice in this experiment were dead by the 21st week. A similar number of mice developed tumours in the other experiment, but after 24 weeks only one mouse survived and this bore a tumour. Particulars of these two experiments together with the other three previously mentioned are given in Table 5. As the death-rate was so high and the number of animals utilized was so small in most of the experiments, only the total tumours produced and the average week before each of the tumours appeared together with the duration of each experiment is given in the table.

Table 5

Agent	No.	TT	AvW	D
Distillate 7 1 %	5	0	—	36 S
Fraction 4 1 %	5	4	18·0	26
Fraction 5 1 %	5	4	27·0	36 S
Distillate 7	25	3	13·0	21
Mixture	25	3	15·0	29

No. = number of mice utilized at the beginning of the experiment; TT = total tumours produced; AvW = average period in weeks elapsing before each tumour appeared; D = duration of experiment in weeks; and S indicates that the experiment is still in progress.

Although these experiments prove fairly conclusively that fractions 4 and 5 are appreciably more carcinogenic than their parent distillate the paucity of available data makes it very difficult to estimate their degree of difference. It would appear that the highly viscous fraction 4 was more carcinogenic than the solid fraction 5, and we decided to perform another experiment with fraction 4 in an effort to determine its approximate degree of activity compared to distillate 7.

The result of this experiment so far indicates that a 1 % dilution of this fraction is about as carcinogenic as a 5 % dilution of distillate 7. Thus fraction 4 would appear to be at least ten times as carcinogenic as oil 46(2) from which distillate 7 was derived. We had not sufficient material available to repeat the experiment with fraction 5, and as a matter of fact we found it necessary to chromatogram a further 300 c.c. of distillate 7 in order to obtain enough of this fraction for the experiment described above. We surmise that fraction 4 contains appreciable quantities of solid material, but owing to its greater solubility is more difficult to separate. This view is strengthened by the fact that recently by adopting a modification of our chromatographic method we have succeeded in obtaining appreciable quantities of crystalline solids from fraction 4 of one of our shale distillates. This fraction yielded no solids when utilizing our original method. Since fraction 4 is apparently appreciably more carcinogenic than fraction 5 the solids present in it, or the viscous oil in which they are dissolved, are presumably on an average more carcinogenic than those comprising fraction 5. From a consideration of the results we obtained with the chromatographic fractions of our shale distillates it would not appear likely that the liquid components of mineral oils, unless highly viscous, are mainly responsible for their biological activity, and we shall not be surprised if it eventually proved that solids or semi-solids are entirely responsible both for their carcinogenic and dermatitic activity. With the modified chromatographic method recently employed we are obtaining large quantities of practically white liquid fractions which appear to be inert or nearly inert, and we expect shortly to be able to separate our oil distillates into about 60–80 % of inert liquid material with the rest semi-solid or solid material of varying activity.

SUMMARY AND CONCLUSIONS

Some concentration of the carcinogenic material in a Venezuelan spindle grade oil has been affected by simultaneous molecular distillation, and further concentration has also been accomplished by subjecting one of the more carcinogenic of these distillates to chromatographic adsorption. Animal experiments revealed that this distillate was about twice as carcinogenic as the oil from which it was derived. Two small fractions obtained from this distillate by chromatographic adsorption were painted on the skin of a few mice and both appeared to be appreciably more carcinogenic than the distillate from which they were derived. Further experiments with one of these fractions and the distillate, not yet completed, indicate that this fraction is at least five times as strong as the distillate from which it was derived. Thus by a combination of molecular distillation and chromatographic adsorption processes we have obtained a fraction about ten times as strong as the original oil. During the chromatographic adsorption process large quantities of colourless, low index, inert or almost inert material of relatively low viscosity have been separated from the highly coloured active remainder. It would appear that the most active material is highly viscous at room temperature, but its activity may be due to the presence in it of crystalline solids.

In conclusion we wish to express our thanks to our steward, Mr L. Norburn, for his valuable assistance in the practical work.

REFERENCE

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