THE REDUCTION OF THE CARCINOGENIC AND DERMATITIC ACTIVITY OF SHALE OIL RECOVERED FROM THE PERITONEAL CAVITIES OF INJECTED MICE

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During the last thirteen years much time and thought has been devoted to the study of mineral oils in connexion with their powers of producing dermatitis and cancer of the skin. As long ago as 1928 Twort & Ing (1928) described methods for freeing oils from their carcinogenic factor. Chemicals employed by them included sulphuric acid, methyl sulphate and sulphite, and also picric acid. Oxidation by means of pure oxygen at 150° C. and reduction by means of amyl alcohol and sodium were also used by them with success.

Later, Twort & Fulton (1929) showed that the carcinogenic action of mineral oils could be considerably reduced by extraction with ethyl alcohol. Later, Lyth (1933) described how the carcinogenicity of oils was related to their refractivity constants and again in 1935 in collaboration with Twort (Twort & Lyth, 1935*a*) showed how the carcinogenicity of oils was related to the fall in their refractive indices when recovered after a period of days from the peritoneal cavities of mice.

The object of the experiments described in this paper was to try and prove that, just as certain chemical treatments which produce a marked lowering of refractive index reduce the carcinogenic potency of mineral oils, so oils, which after recovery from the peritoneal cavities of animals show a pronounced lowering of refractive index, also are less active when applied to the skin of mice.

Oil recovered from the peritoneal cavity of injected rabbits

Some tentative experiments in collaboration with Dr A. Lasnitzki, formerly of this department, and now of the department of Physiology, Birmingham, were performed 2 years ago with a carcinogenic Persian oil (our no. 210). In these experiments 9–13 c.c. of oil were injected into the peritoneal cavities of some twenty rabbits. The method used for recovering the oil from the peritoneal cavity and subsequent treatment of both the recovered and control oil before application to the skin has been described by Twort & Lyth (1935*a*). They (1935*b*) have also published particulars as regards standardization of dose in relation to the weight of the injected animal.

As, usually, oils appear to be more rapidly absorbed when injected into the peritoneal cavities of rabbits than when injected into those of mice, the standard time of 7 days was reduced to 4. The rabbits were therefore killed and the oil recovered 4 days after injection. Unfortunately, even then only traces of oil

were found in about half of the rabbits, the rest yielding an average of 20% of the oil injected. The refractive index of the control oil at 25° C. was 1·5133, and the average for the recovered oil was 1·5071. The average fall in index was therefore 0·0062. There was a variation in fall, however, of about 0·0055–0·0080 in the various samples recovered from the rabbits. The average fall in this oil recovered after 7 days from the peritoneal cavities of four injected mice was 0·0101.

APPLICATION OF OIL RECOVERED FROM INJECTED RABBITS AND CONTROL OIL TO THE SKIN OF MICE

Two experiments were performed with the recovered oil with ten mice in each experiment. Two control experiments, one with thirty mice and the other with ten, were started at the same time. In the first experiments with the recovered and control oil the mice were painted as usual five times weekly on the interscapular region, but in the second experiments, in order, if possible, to reduce the death rate, they were painted only twice a week for some of the time and for the rest of the time five times weekly. However, in spite of this precaution, all the mice painted with the recovered and control oil died in the second experiments before the advent of tumours. In the first experiments paintings continued for 30 weeks when no more recovered oil was available. At this time there were only four survivors from among the ten mice painted with the recovered oil. Two of these bore small papillomas which subsequently disappeared. Of the thirty controls there were at this time only seven survivors, five of which bore papillomas. Two of these mice subsequently died bearing malignant tumours, confirmed microscopically. Of the remaining three, two bore papillomas which disappeared before the mice died, and one died bearing a large non-malignant papilloma.

Throughout the experiments no very obvious differences were noted as regards epilation or the severity of dermatitis, but loss of weight and prevalence of hyaline changes in the internal organs (liver, spleen and thyroid) was somewhat more marked in the controls. The last fact and the production of two malignant tumours by the control oil indicated that there was probably some reduction in potency of the recovered oil, but from the few data available no certain conclusions could be drawn.

OIL RECOVERED FROM THE PERITONEAL CAVITIES OF INJECTED MICE

As a result of the experiments mentioned above, we decided to perform more elaborate experiments with a carcinogenic shale oil. Since from our own experience and that of others (Polson, 1931) we know that rabbits do not tolerate well injections of carcinogenic shale oil, mice were injected instead of rabbits. Moreover, besides recovering relatively more oil, there is generally very little variation in the amount of index fall for a particular oil when recovered from injected mice. The oil used was a refined spindle grade oil having a refractive index at 25° C. of 1·5040. About 350 mice were injected

from time to time with our standard dose (0·5 c.c. for a 20 g. animal). The oil was allowed to remain in the peritoneal cavity for 7 days, after which time the mice were killed and the oil recovered. About 300 of the mice yielded an average of 30% of the oil injected. Of the remainder some died before the seventh day and were not utilized. In others the injections had been misplaced or no oil was found. The recovered oil was subjected to Twort & Lyth's (1935*a*) chemical method for removing saponifiable material, and the control oil was similarly treated before being applied to the skin. No demonstrable change of index was noted in the control oil after such treatment. The average index of the recovered oil was 1·4954.

Application of recovered and control oil to the skin

Fifty mice were treated with the control and fifty with the recovered oil. The oil was applied five times each week to the interscapular region for 22 weeks and subsequently twice a week until the thirty-fifth week, when all the surviving mice were killed.

After about twelve applications it was apparent that the power to produce epilation was much more pronounced in the control oil. Later it was observed that more of the mice treated with the control oil developed dermatitis and, still later, ulcers and tumours were conspicuously more numerous amongst them. The first tumour appeared at the ninth week, but it was not until 3 weeks later that the first mouse developed a tumour in the experiment with the recovered oil. At the end of 24 weeks tumours had appeared in twenty-three of the control painted mice and only six out of the twenty-four survivors were tumourless. At this time only eight tumours had appeared in the mice painted with the recovered oil and out of twenty-five survivors nineteen were tumourless. Moreover, of the total tumours produced throughout the experiments nine of those borne by the mice painted with the control oil were considered malignant, whereas only two among those borne by the mice painted with the recovered oil were classified as such. Macroscopic diagnosis of tumours was in nearly all cases confirmed with the microscope. At the thirty-fifth week only eight mice in the control experiment survived, but there were sixteen survivors in the other experiment. Seven out of the eight control survivors bore tumours, four of which were considered malignant, whereas of the sixteen treated with the recovered oil six only had tumours, one of which was malignant. Particulars as regards number of benign and malignant tumours and numbers of survivors, etc., at 25 and 35 weeks respectively, are given in the following table.

	25 weeks			35 weeks			
	Mice living	T	M	Mice living	T 1	M 1	C.P.
Control oil	22	23	4	8	26	9	122
Recovered oil	23	9	1	16	11	2	27

T and T 1 denote the number of mice both living and dead which bore or had borne tumours at 25 and 35 weeks respectively, and M and M 1 the

number of tumours classified as malignant. The carcinogenic potency figures (C.P.) are derived from the mean of our (Twort & Twort, 1933) methods 1 and 2.

Scrutiny of the table reveals a striking difference in the figures given by the two experiments. It does not matter whether we compare the total tumours, the number classified as malignant, or our potency figures, all give pronounced indications of reduced activity on the part of the recovered oil.

No post-mortems were performed on the mice which died during the first 20 weeks or so of the experiments, but after that time, the skins and sections of livers and spleens of most of the dead animals were examined microscopically. Microscopic examinations of these organs were made in all mice killed at the termination of the experiments. Of the mice examined belonging to the control group, numbering twenty-seven, twenty-one showed hyaline changes in the spleen and nineteen similar changes in the liver; the percentage incidence being 77 and 70 respectively. On the other hand, of the twenty-five examined in the group treated with the recovered oil, eleven revealed hyaline changes in the spleen and eight only in the liver; the percentage incidence being respectively 44 and 32.

DISCUSSION

Although the experiments with Persian oil recovered from the peritoneal cavities of injected rabbits showed only slight reduction in the carcinogenic potency, it seems probable that much more conclusive results would have been obtained had a greater amount of material been available for the painting experiments. Still better results might have been obtained had mice been used instead of rabbits to obtain the necessary material. As pointed out in using rabbits, relatively much less oil is recovered, and it is necessary to recover the oil at the end of 4 days instead of 7. At the end of this time the refractive index of the oil is lowered on an average from 1·5133 to 1·5071, whilst in mice after 7 days it is lowered on an average from 1·5133 to 1·5032. When the experiment was started it was considered that this first drop would be adequate for our purpose, but our results indicate that it is desirable to produce a bigger drop in refractive index before conclusive results can be expected.

The experiments with the shale oil indicate fairly conclusively that not only the carcinogenic potency, but also the factors that produce epilation and dermatitis are considerably reduced in the recovered oil. The lower death rate and the reduced incidence of hyaline changes in the internal organs of the mice painted with the recovered oil give further indications in this direction. The probable processes involved in the reduction of refractive index by the injected animal have been discussed previously by Twort & Lyth (1935*a*). As a result of viscosity determinations on several mineral oils, including shale, with kinematic viscosities varying from 40 to 137, it appears that, at any rate within this range, little change in viscosity is effected by the animal when the oil is recovered after 7 days. The viscosity of the recovered shale oil appeared to be slightly raised. This appeared to be true also of some mineral oils, whilst the viscosity of

others was somewhat lowered. The differences however were never much greater than could be expected as a result of experimental errors. Many experiments performed with mineral oils, including shale, indicate that oil fractions of low viscosity are mainly dermatitic, whilst most of the carcinogenic fractions are more viscous. This would account for the fact that the viscosity of the recovered shale oil differs so little from the control, since, as we have seen from the results of the painting experiments, the animal appears to remove a certain amount of both the dermatitic and carcinogenic material from the oil injected into its peritoneal cavity.

It is of interest to compare the activity of the recovered oil with the activities of some shale oils treated with sulphuric acid and extracted with ethyl alcohol. The index of a shale oil fell from 1·5034 to 1·4957 when treated with about 6% of sulphuric acid. This treated oil when applied to the skins of mice gave a potency of 75 whilst the control oil gave a potency of 293. The residue of another shale oil left after extraction with alcohol gave a refractive index of 1·4940 the index of the control being 1·5039. Here the reduction in potency was from 244 to 36 when applied daily to the skins of mice. We thus see that there is a relationship between the refractive index fall and reduction in potency of shale oil whether this fall is brought about by certain chemical treatments outside the animal body or by the animal itself.

Summary

1. A carcinogenic Persian spindle grade oil recovered after 4 days from the peritoneal cavities of injected rabbits showed indications of reduced carcinogenic activity for the skins of mice.

2. The activity of a carcinogenic shale oil recovered after 7 days from the peritoneal cavities of injected mice was found to be considerably reduced. The reduction in refractive index and potency of the recovered shale oil showed an obvious parallel to that resulting from certain chemical treatments, namely, treatment of the oil with a certain quantity of sulphuric acid or extraction with alcohol.

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