A NEW METHOD FOR MEASURING CARCINOGENICITY

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The well-established experimental fact that repeated contact of certain mineral oils and tars with the skin of animals may lead to cancer production has stimulated investigation on the nature of the process and on the reason why the animal reacts in this manner. In these laboratories many different paths have been explored, but perhaps one of the most promising is that which forms the subject of the present paper. Before we enter into a discussion of the experiments involved it may be well if we first mention briefly the theoretical ideas which prompted us to undertake the particular experiments in question.

The reason why cancer follows the frequent application of a carcinogenic agent to the skin of an animal is unknown, and it is not even known whether the agent acts directly or indirectly. It may act by directly exciting the cell, with which it comes into contact, to undergo that particular type of metaplasis which ultimately will end in cancer formation, or the action may be indirect by exciting the organism to elaborate specific substances which themselves are capable of inducing cancer development. Cancer itself may be the result of repeated stimulation of the cell to divide in order to protect the deeper tissues from the noxious action of the agent by a mechanical thickening, or it may be the results of the body's effort to detoxicate the agent on the spot. These appear to us the outstanding points in connection with the mechanism of the formation of mineral oil cancer which require elucidation.

The present paper deals essentially only with the last mentioned possibility. From our knowledge of infection and immunity in bacteriology, etc., we are aware that the first effort of the body is to localise the harmful material and the ultimate object is to destroy—localisation or elimination, neutralisation or destruction. We have always felt convinced that the body would react in a somewhat similar manner against mineral oils and tars, but it is especially in connection with neutralisation that we are concerned here. We imagined that the animal body would:

(*a*) attempt to neutralise the noxious constituents of the agents, and

(*b*) that this neutralisation would be along the lines of saturation of unsaturated constituents and hydrogenation of not fully hydrogenated constituents.

As we were only in a position to use mice or at the most rabbits, we were unable to perform some of the most obvious experiments such as the feeding of animals and the recovery of the oil from the faeces for subsequent testing on

the skin of mice. We thus decided to work along entirely different lines wherein the quantity of oil required would be a minimum. In every respect this has obvious advantages provided the experimental results ultimately available had an all-round high value.

Our basic test for the degree of neutralisation of the active constituents of mineral oils by the animals is the amount of change in the refractive index of the oil when allowed to remain in the peritoneal cavity of mice for variable periods of time. 0·5 c.c. of the mineral oil to be tested is injected into the peritoneal cavity of one to a dozen mice. The animals are killed at intervals of one week, and the oils recovered by washing out the peritoneal cavity with water. The fluid is centrifuged, the top fatty layer being removed, and treated with 2 per cent. caustic soda, in a steam bath.

The oil is pipetted off and the residue washed several times with toluol; the toluol extract and oil being dried over calcium chloride for 24 hours, and then filtered. The toluol is evaporated off in an oven at about 60° C., and the refractive index of the oil read after keeping the oil for a week in the oven. The oil is kept in the oven until two consecutive readings are similar. If sufficient oil is only available for one reading it is well to delay the examination for at least three weeks. In each test control uninjected oil is tested and examined in a parallel manner.

We argue that:

(*a*) The higher the carcinogenicity the greater the lowering of the refractive index. (N.B. not necessarily the higher the degree of unsaturation or the higher the degree of dehydrogenation.)

(*b*) Females should react slightly more than males.

(*c*) Albino-eye animals should react slightly more than pigmented eye animals.

(*d*) Early tumour bearing animals should react slightly more than late tumour bearing animals.

(*e*) Large animals should react more than small animals of the same variety.

(*f*) Mice should for some reasons react more and for some reasons less than rats, weight for weight.

We intend to deal here only with hypothesis (*a*). A batch of 140 animals received an intraperitoneal injection of eighteen different mineral oils with varying degrees of carcinogenic activity. The amount injected was in almost all instances 0·5 c.c., but even this quantity of some of the oils was not tolerated by the animals, for they died within 24–48 hours after the injection. The most toxic oils were Nos. 49, 69 and 113, two Borneo and a Roumanian oil respectively, only two animals out of thirty surviving for 48 hours. There remained, including oil 113, sixteen oils, two of which were tested twice, making in all eighteen experiments available for correlation purposes. The total number of specimens of oil recovered from the peritoneal cavity was sixty-one, taken

from 1–10 weeks after the injections. The average fall in the refractive index (R.I.) each week was:

Week	No. of specimens	Average fall in R.I.
1	18	0·0059
2	15	0·0021
3	11	0·0015
4	9	0·0003

the fall during subsequent weeks being negligible. The highest fall was 0·0179, and the lowest 0, or a rise of 0·0004.

For correlation purposes the simple ranking method of correlation was used, where $C = 1 - \frac{6 \text{ Sigma } d^2}{n(n^2-1)}$, perfect correlation giving 1.

The carcinogenic potency (P.), fall in refractive index (R.I.F.) during the first week, and refractivity constant (R.C.) were then correlated one against the other, with the following results:

P. and R.I.F.	0·752
P. and R.C.	0·674
R.I.F. and R.C.	0·586

The first figures are above the others chiefly on account of a Persian cylinder oil, which had, of course, a low P. but a high R.C., the fall in the refractive index being the lowest registered with the exception of that of liquid paraffin. The original refractive index of the latter oil was 1·4824, and it presumably remained unchanged in the animal body, the figures we obtained from the first to the 10th week being:

Week	R.I.	Week	R.I.
1	1·4824	6	1·4824
2	1·4827	7	—
3	—	8	1·4826
4	1·4825	9	1·4826
5	1·4824	10	1·4828

These results give one an idea of the amount of error probably to be expected, the tendency for there to be a slight rise in some instances being presumably due to experimental error. If we take the R.I.F. figures of the oil obtained from the last animal instead of the first animal of an experiment and correlate with carcinogenic potency we obtain a correlation coefficient of 0·781, slightly better than before, although the length of time the different oils had been in the peritoneal cavity of the animals varied considerably.

Our experiments show conclusively that in general terms the greater the reduction in the refractive index of the oil the greater the carcinogenicity of the original oil for the skin of the particular animal in question. They do not, however, prove that the greater the reduction in the refractive index the more intense the reaction of the animal because some oils will presumably require a much more intense reaction on the part of the animal than do other oils, in order to bring about a similar drop in the refractive index. Nevertheless, for the sake of simplicity, it would appear best meanwhile to assume that the

greater the carcinogenicity of an oil for a given animal the greater the reaction of this animal, the latter being measured more or less accurately by fall in refractive index.

It is interesting to note that while animal reaction appears to be directly proportional to carcinogenicity it is certainly not proportional to the degree of unsaturation of the oil if other things, such as viscosity, boiling range, etc., are not equal. In this respect the test is similar to that of the refractivity wherein viscosity and boiling range have to be taken into account when estimating the probable carcinogenic activity of an oil. Our results open up a wide field of research. One of the most important questions to be answered is whether the drop in refractive index is indicative of a chemical change in the constituents of the injected oil such as oxidation or reduction, or whether it is due simply to removal of selected constituents of the oils from the peritoneal cavity, the removed constituents being in effect those responsible for the production of skin cancers, *i.e.* the unsaturated or partially dehydrogenated substances within a particular boiling range.

If we assume that the density of the oils is unchanged we can find the fall in the refractivity of each oil, and again correlate with the carcinogenic potency. We found the correlation coefficient here to be 0·793 as against 0·781 found for the same oils when the fall in the refractive index was utilised. A scrutiny of our figures indicated that there was probably some relation between the viscosity of the oils and amount of change which takes place in the oil while in the animal body. We know that there is a relation between the viscosity and carcinogenic potency, and in the particular group of oils under discussion the correlation coefficient proved to be 0·111, a low viscosity indicating a high carcinogenic activity, other things being equal. On the other hand, viscosity correlated with fall in refractivity and fall in refractive index gave much higher figures, viz. 0·427 and 0·423 respectively, the animal apparently reacting more easily to oils of low viscosity than to those of high viscosity. We may recall that it is the latter oils, on the contrary, which we have found to be chiefly instrumental in producing fatty infiltration (condition *X*) of the liver.

The fall in the refractivity, on the assumption that density is unchanged, was, with the exception of a cylinder oil and liquid paraffin, considerable; many of our figures being far below that of the latter oil. The three lowest figures were found with three oils which we believe to have a high general toxicity for mice, irrespective of their carcinogenicity for the skin of these animals. It is of course improbable that the density remains entirely unchanged, but in any case it is essential to discover the degree of this change so as to gain an idea of the extent, if any, of the chemical alteration in the constituents of the oils when injected into animals.

We may mention meanwhile a few experiments bearing on this question, which were performed several years ago when examining the nature of the substances apparently responsible for the fatty infiltration (condition *X*) of the liver following skin applications of mineral oils. By taking 20–50 mice, painted

for 6–12 months with mineral oils, it was possible to extract from the massed livers a minute quantity of an unsaponifiable oily substance, whereas the livers of animals painted with cod-liver oil yielded no fluid extract. These were very laborious experiments and for the time being were discontinued. We were able to recover oils from three lots of liver in sufficient quantity for a refractive index reading, the following being the figures found:

Oil No.	Original R.I.	R.I. of liver extract
55 Shale	1·5054	1·4916
58 Russian	1·4950	1·4900
144 Pennsylvanian	1·4825	1·4886
143 Cod Liver	1·4787	No extract

It will be noted that with the first two oils there is a fall and with the third there is a rise in the refractive index so that the figures for the three extracts approximate one another closely although the higher the original index the higher that of the liver extract. This may indicate that when the oils are removed from the back of the animal, and ingested, it is only certain specific constituents that are able to be assimilated by the intestinal tract, and eventually to be deposited in the liver. These results are in favour of a chemical change in the oils injected into the peritoneal cavity, as being responsible for the fall in the refractive index, and not the selective removal of certain specific compounds. We have here a possible explanation for our inability to produce tumours in mice subjected to a single injection of mineral oil or even very highly carcinogenic synthetic tars. We have injected some 750 animals by various channels but have not been able to record a single success.

SUMMARY

Mineral oils recovered from the peritoneal cavity of injected mice showed in all sixty-one instances, with the exception of liquid paraffin, a fall in the refractive index. This phenomenon is presumably due either to a chemical change (? oxidation or reduction) in the constituents of the oils brought about by the juices of the animal organism, or to a mechanical change resulting from the removal of selected constituents to other parts of the animal. This test will possibly prove to be superior to that of skin applications[1] and estimation of the refractivity constants[2] for measuring the amount of carcinogenic constituents in mineral lubricating oils and it has the advantage of necessitating the use of only one animal instead of 100. We are of opinion that our experiments may be of far-reaching importance. They carry us a definite step nearer to the understanding of the mechanism of cancer formation.

[1] (1931), *J. Industr. Hygiene*, **13**, 204.
[2] (1933), *J. Hygiene*, **33**, 464.

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