THE VIOLENCE OF REACTION OF THE ANIMAL IN RELATION TO THE ETIOLOGY OF CANCER AND INFLAMMATION

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(With 1 Figure in the Text)

We recently described a new method for measuring the carcinogenicity of mineral oils, etc. (*J. Hygiene*, **35**, 125). Further researches have provided a considerable amount of fresh data, which are briefly discussed in this paper. It may be recalled that the basic principle of the test depends upon the fall in the refractive index of an oil when injected into, and subsequently recovered from, the peritoneal cavity of the experimental animal. The standard procedure we have provisionally adopted is the injection of 0·5 c.c. of oil into a 20-g. male albino mouse, the oil being recovered after remaining 1 week in the animal. Up to the present we have performed about 800 tests with some 200 oils, half of which were ordinary commercial products. The general indications we have obtained are that the fall in the refractive index (R.I.F.) is proportional to the degree of unsaturation or dehydrogenation of an oil of given viscosity, the fall being a measure of the carcinogenicity plus dermaticity of the oil in question.

In the first place we shall consider the carcinogenic potency of the oils, our records being analysed from the point of view of correlation coefficients obtained by the simple ranking method, perfect correlation giving unity. Here we found that the correlation of the first 100 ordinary commercial products tested on the skin and injected into the peritoneal cavity of animals gave:

Carcinogenic potency and refractive index fall	...	0·616
Carcinogenic potency and refractivity constant	...	0·535
Refractive index fall and refractivity constant	...	0·472

so that with these particular oils it appears that the refractive index fall after injection gives a better indication of their carcinogenicity than does an examination of their refractivity constants. A further group of 60 oils obtained by treating some of our samples with solvents, etc., gave for carcinogenic potency and R.I.F. a correlation coefficient of 0·638, but data as to refractivity constants were here unfortunately not available.

It may be pointed out that our figures would be considerably improved were certain precautions taken in the selection of specimens. The 160 oils referred to above were random samples, and we were aware that, owing to poor

survival rate among the particular batches of animals concerned, many of the potency figures allocated to certain of the oils were very dubious. Also, no correction being made for viscosity, our figures were bound to be adversely affected. The R.I.F. varies inversely as the viscosity, as does to a less extent the carcinogenic potency within a particular viscosity range. The correlation coefficients we obtained with our 100 commercial oil samples were:

Refractive index fall and viscosity	...	...	−0·531
Carcinogenic potency and viscosity	...	...	−0·190
Refractivity constant and viscosity	...	...	−0·104

The only data regarding viscosity which we have meanwhile available are those provided by the suppliers of the oil samples, but as in commercial practice it is not necessary for physical characteristics to be very exact we are ourselves examining our oils as to the absolute viscosity units of each sample. The R.I.F. of a mineral oil being presumably a measure of its carcinogenic plus dermatitic constituents, it is possibly only by the application of a viscosity correction that we shall be enabled to segregate the former from the latter.

That viscosity *per se* plays little or no part in the R.I.F. can, of course, be easily shown. Two series of animals were injected with a 50—50 blend of oil 102 (R.I.F. 0·0137) and oil 124 (medicinal liquid paraffin, R.I.F. 0·0000), and with a blend of oil 102 and oil 243 (slab oil, R.I.F. 0·0000), the viscosity of oil 102 being thereby raised and lowered respectively. In the former case the average R.I.F. was 0·0075, and in the latter 0·0077. Again, we have found all mineral oils of a fair density of colour, with a Redwood No. 1 viscosity up to 2000 at 70° F., to give a definite R.I.F. when injected into animals. When, however, a cylinder stock oil of zero R.I.F. is blended with our slab oil, also of zero R.I.F. so as to have a mixture with a viscosity below 2000 at 70° F., the mixture gives an R.I.F. of zero when injected into animals. A variety of experiments with oil blends shows that the animal reacts exactly according to the task allotted to it, the smaller the quantity of oil injected the greater the R.I.F., and the more an active oil is diluted with one of lower activity the greater the R.I.F. of the active oil. An illustration of our meaning is given below:

Oil 124 c.c.	Oil 102 c.c.	R.I.F.
0·500	0·000	0·0000
0·475	0·025	0·0015
0·425	0·075	0·0025
0·375	0·125	0·0040
0·325	0·175	0·0055
0·275	0·225	0·0065
0·250	0·250	0·0075
0·000	0·500	0·0137
0·000	0·250	0·0170

It is important to have information of any change in the density, and to a less extent of the viscosity, of the injected oils. Apparatus for density measurements of small quantities of oil has recently been acquired, and it is hoped thereby to obtain further indications of the mechanism of the change which

the oils are undergoing in the animal, and also possibly to be enabled to introduce a second correction factor so as to improve our correlation figures. The density of a single sample of recovered oil has already been ascertained, and this, as naturally anticipated, showed a considerable drop in the density although, again as anticipated, not in proportion to the fall in the refractive index. Consequently the refractivity constant of the oil in question was lowered, and it is to be presumed that other mineral oils will act similarly, the fall in refractivity being directly proportional to the carcinogenicity plus dermaticity.

We have repeatedly pointed out that the general toxicity of mineral oils for animals painted with them, as measured by fall in weight and death-rate, is roughly proportional to their carcinogenic activity or to their ability to induce dermatitis. This is well exemplified if instead of being painted on to the skin of the animal the oil is injected into the peritoneal cavity. The correlation coefficients found for our 100 oils were:

R.I.F. and fall in weight of animal...	0·532
Carcinogenic potency and fall in weight of animal ...	0·399
Refractivity constant and fall in weight of animal ...	0·223
Viscosity and fall in weight of animal	−0·421

Again, as we have stated in previous publications, when animals are painted with mineral oils over a fairly long period of time a curious form of fatty infiltration (condition *X*) of the liver may supervene, there being apparently an absorption of the oil through the intestinal mucosa. The recovered oil may show a refractive index fall or rise, according to the index of the original oil. Condition *X* is probably due to the saturated constituents of the oil, and its intensity varies directly with the viscosity. On the other hand, hyaline degeneration of the organs, which may supervene after painting with mineral oils, is probably due to the unsaturated constituents, and its intensity is apparently inversely proportional to the viscosity. In the early days of our investigations of the animal reaction to applications of mineral oils we observed that there was apparently an antagonism between hyaline degeneration of the organs and condition *X* of the liver, but we did not appreciate at the time that this apparent antagonism was really due to a difference in constitution of the particular oils utilised.

In order to make clear our present conception of the reaction of the animal to the different constituents of our oils (in all cases, with the exception of condition *X*, due to the unsaturated or aromatic constituents) we have drawn up a chart. It must be understood that it is only very approximate and in minor respects is based upon no experimental proof. On the other hand, it enables one to visualise without difficulty the essential features of the animal reaction and when and why they are to be expected.

It will be gathered from the chart that the cancerous constituents mostly boil at from 250 to 350° C., while the dermatitic mostly boil at about 200° C. below this. Loss of body weight increases as the boiling range is lowered until

the range of 100° C. is reached, when the animal will probably die immediately after injection. Hyaline degeneration of the organs and R.I.F. of the oil follow closely loss of body weight, although we have not so far been able to show for certain that the indices of the very lowly boiling constituents are changed. It will be noted that the highest boiling unsaturated and aromatic constituents are apparently inert in all respects, as also are all hydrogenated constituents with the exception that they lead to condition *X* of the liver. The low boiling constituents from any crude oil presumably kill by their solvent action on lipoids, etc.

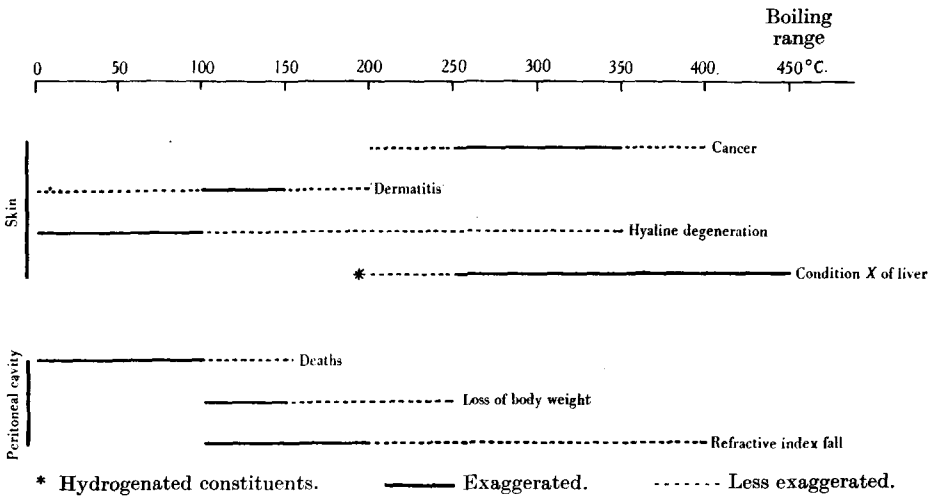

Fig. 1. The animal reaction to the different constituents of mineral oils.

It has already been mentioned that the amount of oil injected into the peritoneal cavity is usually 0·5 c.c., a mouse of about 20 g. weight being chosen. We have found of course that:

The longer the survival time of the animal the greater the R.I.F.
The greater the body weight (w) of the animal the greater the R.I.F.
The smaller is w the greater is the R.I.F. per gramme w.
The smaller the quantity of oil injected (Q) the greater the R.I.F.
The smaller are w and Q in the constant $\frac{Q\ (0{\cdot}5\text{ c.c.})}{w\ 20\text{ g.}}$ the greater the R.I.F.

The dose of oil for injection must not be directly proportional to the body weight, but must more nearly vary with the square of the cube root of the body weight, 0·5/7·37 being our constant. The following injections were made for the purpose of comparing the reaction of the rat with that of the mouse.

Rat of 200 g. injected with 2·5 c.c. of oil 102 R.I.F.	...	0·0118
Rat of 200 g. injected with 5·0 c.c. of oil 102 R.I.F.	...	0·0094
Mouse of 20 g. injected with 0·25 c.c. of oil 102 R.I.F.	...	0·0170
Mouse of 20 g. injected with 0·50 c.c. of oil 102 R.I.F.	...	0·0138

Our results gave a false impression of a lower degree of reaction to the oil on the part of the rat compared with that of the mouse. The doses for comparing a 200 g. rat with a 20 g. mouse should have been 2·3 and 0·5 c.c. respectively, and we then find that if there is a quantitative difference in the reactivity of the peritoneal cavity of these two animals it is not comparable to the difference appertaining to the skin. In this connection it may be mentioned that meanwhile we have been unable to detect any difference in reactivity of our mice related to sex or colour of coat and eyes when the animals are injected with oils, although we had previously been able to demonstrate a difference as regards skin susceptibility. In the latter case, however, we were dealing with many thousand animals. (*J. Hygiene*, **32**, 557).

The relation of susceptibility of the peritoneal cavity to susceptibility of the skin is being tested in another manner, the amount of fall of the refractive index of injected oils being compared in the tumour- and the non-tumour-bearing animals of an experiment. Some 200 animals have so far been injected for this purpose, but no definite indications as to the point in question have yet been observed. A still further line of investigation is being resorted to, viz. that of the effect of saponifiable oils in interfering with the peritoneal reaction, and consequently with the fall in the refractive index of injected oils. Our results here are meanwhile not sufficiently clear to warrant discussion.

It may be mentioned that squalene, an unsaturated pure hydrocarbon oil with a very high refractivity (obtained from the liver of the dog-fish), appears to be totally inert when injected into animals, the refractive index remaining stationary. Blended with textile mineral oils it seems to act as a passive diluent in the same way as do liquid paraffin and slab oil. It is to be presumed, however, that even the most inert of oils have, by virtue of their oiliness, a retarding influence on the animal reaction against carcinogenic agents both as regards skin and internal reactions.

On injection of oils into the peritoneal cavity the reaction of the animal, accompanied by an invasion of leucocytes into the cavity, is roughly proportional to the amount of unsaturated and aromatic constituents in the oil. If to the emulsion recovered from the cavity a little fat stain is added, and the mixture, after shaking, is centrifuged, the difference between the tubes containing a saponifiable oil and one containing a mineral oil is at once evident. In the latter case the leucocytic deposit is hardly tinged with the stain, the bulk of which is retained by the thick layer of free, surface-swimming oil. Microscopically the conspicuous intracellular oil globules, present, for example, when olive oil is used, are absent in the case of mineral oils. The possibility of the leucocytes playing a part in the chemical change and removal of the mineral oil led us to attempt to accelerate the reaction by exciting an artificial leucocytosis by the previous injection of broth into the peritoneal cavity. Apparently the role of the leucocytes is unimportant, as it was found that the average animal receiving broth reduced the index of the subsequently injected oil to a slightly less extent than the average control animal. The endothelial

cell proliferation which later leads to local trapping of the oil, and eventually to a condition of pseudolipomatosis of the whole peritoneal lining of the cavity, would seem to play a much more important part.

On the injection of carcinogenic oils into the subcutaneous tissues there is also an intense reaction, with invasion of leucocytes, the latter eventually disappearing and leaving a sac of oil. After remaining for a few days in the subcutaneous tissues the R.I.F. of the oil is only about one-tenth of that recovered from the peritoneal cavity.

The process involved in the reduction of the refractive index by the animal has not meanwhile been definitely established, although there are strong indications in a particular direction. From the practical point of view of the diagnoses of the carcinogenicity and the dermaticity of mineral oils this question is of no importance, but from the point of view of our understanding the mechanism of cancer it is absolutely essential that the process should be very closely studied. There are four obvious possibilities: chemical change, permeation of cell membrane, local trapping by the endothelial tissue and removal to other parts of the body. In our opinion the indications are strongly in favour of the essence of the phenomenon being due to chemical change, for although we are aware that local trapping takes place, no oil finally remaining free in the cavity, the trapped oil has an index fall as great or even greater than that of the oil remaining free in the cavity. There appears to be a striking analogy between the way in which the animal deals with the oil, and the way in which we deal with it in the laboratory when utilising extractives or sulphuric acid. In all instances we have a lowering of refractive index, density, refractivity and probably viscosity. In other words, the physical properties of an oil recovered from a mouse inoculated one week previously will be closely similar to those of the same sample of oil treated with about 5–20 per cent. of pure sulphuric acid. In spite of our knowledge of the capabilities of the animal organism in performing difficult *in vitro* tasks, the present instance seems to us extremely remarkable. If the R.I.F. is primarily due to a chemical change in the constituents of the oils it is to be presumed that there would be a relation between the amount of the fall and the rapidity of metabolism of the injected animal. We are attempting to effect the metabolism of the animal by the injection of thyroxin, and by maintaining the animals in the heat and cold, and it is also intended to inject animals other than mice and rats.

However much in doubt one may be as to the exact nature of the process involved in the change in constitution of the injected oil, one can have little hesitation in linking it up with the cancer process itself. The correlation coefficients, to our mind, speak for themselves. Although it may be argued that our results are really indirect, since the dermatitic constituents are always more or less associated with carcinogenic and it is only by virtue of the reactive former that we are enabled to assess the amount of the latter, we think this explanation to be ill founded. It seems almost a platitude to point out that were the animal reactionless to the carcinogenic constituents of an oil such an

oil could not induce cancer. A far more likely explanation would appear to be somewhat as follows. Other things being equal the smaller the molecule, or in general terms the lower the boiling-point or viscosity, the more easily is the animal able to deal with this particular constituent of an oil, and consequently the more violent is the reaction. The indications thus are that the difference between dermatitis and cancer is really only one of difference in violence of reaction on the part of the animal, and the reason why there is a falling off in the incidence of cancer when a boiling range above 350° C. is reached is because the reaction is here so sluggish that the average animal does not, so to speak, develop cancer until after it is dead. We have many observations which indicate that in all probability our contention is correct. For instance, a series of distillates of a straight shale oil induced, when painted on to animals, a large number of benign tumours and but a few malignant with the low boiling fraction, while the percentage of tumours becoming malignant gradually increased as the boiling range became higher.

Again the R.I.F. of shale oil is badly out of place, the peritoneal reaction being less than the observed carcinogenic potency required for the skin. While this may be due to the very different constitution of shale oils to that of petroleum oils obtained from wells, it appears at least partly to be due to the relatively small amount of dermatitic constituents in the former. This is deduced by injecting a series of animals with shale oil and a second series with an ordinary petroleum oil giving an R.I.F. similar to the shale oil in 10 days, but having a very much lower carcinogenicity. An animal from each series being killed each day and the R.I.F. plotted against the time the oil has been in the peritoneal cavity, the shale oil graph was found to be nearer a straight line than the control graph, indicating a delayed change in the former oil. Further, weakly carcinogenic agents can be divided into two quite distinct categories: (1) those which induce much dermatitis with early papillomas having a long, or even infinite malignant lag, a good example being turpentine or some polishing oils; and (2) those which induce a small amount of dermatitis with late papillomas, having a short malignant lag, a good example being chrysene.

We have examined the peritoneal reaction to oils oxygenated at different temperatures, having shown in some of our early experiments that there is a gradually decreasing carcinogenicity as the temperature utilised is raised. While we have no information as to changes in viscosity and density of oils so treated, although from inspection we believe the former to be raised, there is considerable change in colour, they becoming darker as the temperature is raised. On account of this darkening the readings of the refractive indices are only approximate, but there are indications of a slight rise above that of the original oil. When injected into animals the R.I.F. was not so marked as that of the control oil of high carcinogenicity, although it was greater than it should have been according to theoretical requirements.

To sum up, it would appear that the road along which the cancer cell is ultimately to travel is paved by the body's persistent effort to neutralise the

toxic influence of the carcinogenic agent, this neutralisation probably being effected by an oxidation or reduction process. In the event of neutralisation being easily performed, the reaction being consequently violent, inflammation only will result, while if neutralisation is a task of extreme difficulty for the animal inflammation will be entirely absent, and cancer will not supervene unless the animal reaches a great age. If the mechanism of cancer is in any way related to the process we visualise then it is highly probable that the mechanism of certain physiological reactions is similar. It has been proved, according to the reports of many different workers, that certain hormones are capable of inducing cancer in animals, and, on the other hand, that certain pure carcinogenic hydrocarbons, related chemically to the said hormones, are capable of exciting the physiological changes normally performed by these hormones. There is thus no far-reaching speculation in deducing that the mechanism underlying both processes is in all probability fundamentally similar. Our ideas on the etiology of cancer (*Lancet*, 1932, ii, 776), the carbon skeleton of phenanthrene being associated with the carcinogenic agent, with sensitisation by unsaturated fatty acids, seem to have additional support from the present experiments, the unsaturated fatty acids probably acting as oxygen carriers.

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