

THE FATTY INFILTRATION OF THE LIVER OF MICE RESULTING FROM THE INGESTION OF MEDICAL LIQUID PARAFFIN, ETC.

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IN a previous paper (1932: *Lancet*, i, 448) we discussed a peculiar type of fatty infiltration of the liver of mice which followed the ingestion of mineral oils. The condition was called condition X as we had no knowledge of the mechanism underlying the process nor of the nature of the fat involved, *i.e.* whether saponifiable or hydrocarbon. We propose now to describe some further observations which we have made on this subject, these more recent observations being related to an entirely fresh group of about 1500 animals. The effect of medicinal liquid paraffin on the organs of mice has been specially studied, and when the condition is advanced it may be visible to the naked eye.

INCIDENCE IN RELATION TO COLOUR OF COAT AND EYE OF ANIMAL.

We had already shown (1932: *J. Hygiene*, 32, 557) that among our animals there was a slight difference in susceptibility to our carcinogenic agents, according to the colour of the coat or eye of the animals. As a matter of fact we now segregate our animals into 20 colour classes, but it will suffice for our present purpose if we divide them only into two groups in two different ways.

Thus: Group 1 *a*. Self-coloured animals (except pure white).

„ 2 *a*. Piebald animals (any colour and white).

Group 1 *b*. Animals with pigmented eyes.

„ 2 *b*. Animals with albino eyes.

As regards the induction of cancer of the skin, we found that Group 1 *a* animals were on an average less sensitive to our tars than Group 2 *a* animals, as were Group 1 *b* less sensitive than Group 2 *b*. In the case of animals painted with mineral oils Group 1 *a* animals were more sensitive than Group 2 *a* animals, while Group 1 *b* were again less sensitive than Group 2 *b*. The most remarkable feature of these findings is the reversal of sensitiveness of the Groups 1 *a* and 2 *a* according to whether one paints the animals with mineral oils or tars.

When we came to examine our animals as to incidence of fatty infiltration of the liver we could only deal with mineral oil animals because the tars as used by us do not induce condition X. In Table I will be found the results

we have obtained with animals painted with miscellaneous hydrocarbons, saponifiable oils, and fatty acids.

Table I.

Group	Number of animals	Number with condition X	%
Self coat	393	154	39.2
Piebald coat	346	134	38.7
Pigmented eye	828	332	40.1
Albino eye	399	107	26.8

It will be observed that there is little apparent difference between the self and piebald groups, but that there is a manifest difference between the pigmented eye and the albino eye groups. We shall thus not consider further the grouping of the animals into selfs and piebalds, but only those with and without pigment in the eye. Having obtained definite indications as to condition X frequency we proceeded to narrow down our animals so that the experimental conditions were as nearly as possible similar. This left us with about 445 animals all of which had been painted for at least 35 weeks with selected mineral oils, and which had been killed at the termination of the experiments concerned. The percentage of animals showing fatty infiltration of the liver was pigmented eye 58.4 and albino eye 43.2, the figure being high in both cases, and consequently the difference small. In sum our analysis shows that animals with pigment in the eyes are more liable to develop condition X of the liver than those without pigment in the eyes; or perhaps it would be better to say that in general pigmented eye animals show the condition earlier than albino eye animals.

INCIDENCE IN RELATION TO SEX OF ANIMALS.

We have not meanwhile satisfied ourselves that there is any difference in susceptibility to our carcinogenic agents between the two sexes, but there is unquestionably a great difference in the incidence of fatty infiltration of the liver. Thus 1118 animals divided into 648 males and 470 females gave respectively 30.1 and 40.4 cases of fatty infiltration of the liver. When the male and female animals were each separated into those with and those without pigment in the eye it was found, as was to be expected from the results of our analysis of pigmented eye versus albino eye, that the pigmented eye females were easily the most sensitive and the albino eye males easily the most resistant. The actual figures we obtained were as under.

Table II.

Eye	Sex	Number of animals	Number with condition X	% with condition X
Pigmented	Males	434	149	34.3
	Females	323	145	44.9
Albino	Males	214	46	21.5
	Females	147	45	30.6
Total	Males	648	195	30.1
	Females	470	190	40.4

When we considered, as before, only animals of selected mineral oil experiments of at least 35 weeks' duration, which were killed at the termination of the experiments, we had similar results as regards sex, all percentages again being higher and the differences somewhat smaller than those given in Table II. Thus the essential fact to retain is that, broadly speaking, the pigmented eye females will give approximately 50 per cent. of cases of the condition while the albino eye males are giving only 25 per cent.

INCIDENCE IN RELATION TO OTHER FACTORS.

In our previous papers we have pointed out several of the factors which may influence the incidence of fatty infiltration of the liver, but our results may be worth while summarising as we have examined a large number of additional animals as well as several other aspects of the subject.

Our chief findings may be summarised as under:

(1) The variations among individual mice due to unknown intrinsic factors are far greater than the variations noted as regards colour and sex.

(2) Rabbits and, we believe, rats are among other species of animals which may develop condition *X* of the liver under similar conditions to those appertaining to mice.

(3) The duration of the time to which the animals have access to the agent is of prime importance, the condition being progressive.

(4) Among organs other than the liver the suprarenal is perhaps the most likely to show fatty infiltration as a result of ingestion of mineral oils. The suprarenal is, as a rule, affected much later than the liver, but when the former organ is affected the latter organ may show little or no evidence of the condition.

(5) Less than 1 per cent. of animals painted with various, mostly dilute, synthetic tars showed the condition.

(6) In one instance only have we observed the condition among animals painted with saponifiable oils, this being in a mouse painted with cotton-seed oil daily for 100 weeks.

(7) Oleic acid definitely induces the condition, although less active than mineral lubricating oils. Painted for 60 weeks, 50 per cent. of the mice may have well-marked fatty infiltration of the liver.

(8) Mineral lubricating oils are the most active agents we have tested. With suitable oils 90 per cent. of mice will have condition *X* of the liver if there is daily access to the oil for 60 weeks.

(9) Saturated mineral oils (medicinal liquid paraffin) appear to be as active as highly unsaturated mineral oils (shale spindle oils—Carcinogenic).

(10) Extracts obtained by treatment of mineral oils with methyl sulphite,

picric acid, sulphur dioxide, acetone, methyl and ethyl alcohol, castor oil, etc., are definitely less active than the original oils. About 30 per cent. of mice may show the condition after access to the extracts for 60 weeks.

(11) The residues remaining after having performed the above extractions are somewhat more active than the original oils. The difference between the activity of the extracts and residues is best shown at about the 30th week of an experiment. At this time 30 per cent. of the livers of the residue animals and less than 2 per cent. of the extract animals will be affected.

(12) Crude oils are on the whole somewhat less active than refined lubricating oils.

(13) On distillation of a crude oil the so-called spirits appear to be practically incapable of inducing fatty infiltration of the liver, while the residues left in the still have but little activity in this direction. The middle boiling range of mineral oils is thus presumably the most active, but we have not meanwhile ascertained the exact boiling range wherein the bulk of the active constituents is located.

(14) Cracked oils are relatively inactive. In a recent experiment a cracked Texas engine oil failed to induce the condition among 12 animals killed after contact with the oil for 45 weeks.

(15) An analysis of our animals indicates that skin tumours have no influence on the production of condition *X* of the liver, and similarly the pathological process in the liver seems to have no influence on the incidence or march of the skin tumours (see later).

(16) Ulceration of skin tumours has also no apparent relation to fatty infiltration of the liver.

(17) Hyaline degeneration inhibits the development of fatty infiltration of the liver (1932: *J. Path. and Bact.* **35**, 219). The hyaline change may or may not be associated with the liver itself. The effect of hyaline on the fatty infiltration is partly shown in Table III.

Table III.

Eye class	Sex	Condition <i>X</i> of liver %	Hyaline degeneration	
			Liver %	Spleen %
Pigmented	Females	44.9	29.1	43.6
	Males	34.3	31.3	46.1
Albino	Females	30.6	32.0	46.9
	Males	21.5	36.9	49.8

It will be observed that as the incidence of fatty infiltration of the liver decreases hyaline degeneration of both the liver and the spleen increases. It will be observed also that the incidence of hyaline changes in the liver is less than that in the spleen, from which facts we foresee the possibility of the fatty conditions of the liver exerting a controlling influence on the hyaline

changes. We imagine that condition *X* of the liver is indicative of an effort on the part of the animal body to deal with the hydrocarbon oil, and as a matter of fact we anticipated the findings of a relatively high incidence among pigmented animals and a relatively high incidence among females. As far as pigment is concerned our findings were in accordance with difference of skin sensitiveness to our carcinogenic mineral oils. Our researches in the field of aetiology of breast cancer, etc. (1932: *Lancet*, ii, 776) led us to believe that females would re-act to mineral oils better than males, and our observations on the incidence of fatty infiltration of the liver support this belief. As we have already remarked we have meanwhile no definite indications that there is a variation in cancer susceptibility to our agents according to the sex of the animal, although we predict that a larger collection of data will show the females to be slightly the more sensitive sex under one condition and slightly the more resistant under another.

We cannot meanwhile see clearly that the fatty infiltration of the liver and the hyaline degeneration of the organs play any direct part in the cancerous process, although we feel that there is somewhere a connecting link between all three processes. This will be appreciated from our previous discussion on the aetiology of breast cancer, and we have made many other observations which lead us to believe that the three processes are not entirely disconnected. The reversal of relative sensitiveness of our animals to mineral oils and tars, according to the colour of the coat of the animal, is in itself a somewhat curious finding, although by no means the most curious. There is a similar reversal as regards hyaline degeneration of the organs, and it is to be noted that while tars are highly carcinogenic they induce practically no fatty infiltration of the liver. Observations on the thyroid gland enlargement and swelling of the pituitary gland following the application of tars and oils also indicate reversal phenomena. Thus in the examination of over 1000 pituitary glands of mice we have observed that the swelling of the gland following the application of carcinogenic agents was more manifest among self-coloured animals than among piebald animals, a finding which we expected according to the skin susceptibility of the animals to the agents, and also their relative susceptibility to the transmissible sarcoma 37/*S*. But the fawn animal which was also relatively sensitive to our agents, although relatively resistant to the 37/*S* tumour, appears to have a pituitary which reacts as that of the average animal to our agents. Again, there appear to be indications of a seasonable variation in the size of the pituitary gland according to sex and colour of the animal, in each case there being a reversal in summer and winter.

SUMMARY.

The fatty infiltration of the liver which follows the ingestion of medicinal liquid paraffin and many other varieties of mineral oils is more prevalent among females than among males, and is more prevalent among pigmented

eye animals than among albino eye animals. When access to the oil lasts for about 30 weeks some 40 per cent. of pigmented eye females and 20 per cent. of the albino eye males may show the condition.

It is the intermediate products, lubricating oils, obtained by distillation of a crude mineral oil which contain the bulk of the constituents which lead to fatty infiltration of the liver, while it is the so-called "spirits" and the residues left in the still which tend to lead to excessive hyaline degeneration of the organs in general. There is evidence to show that fatty infiltration of the liver and hyaline degeneration of the organs are mutually antagonistic, but there is little evidence to show that either condition is directly related to the cancerous process.

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