CANCER SUSCEPTIBILITY IN RELATION TO COLOUR OF MICE.

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A few years ago we instituted some breeding experiments with mice in order to ascertain whether there was any difference as to cancer susceptibility to our agents according to strain. Our method was to mate the first male and female to develop a tumour in a particular experiment, and the last surviving healthy male and female which did not develop a tumour. In this way we hoped to cultivate strains of widely different susceptibility, the progeny of the first tumour animals and the refractory animals being selected from consecutive experiments, each involving one generation. Unfortunately, we were obliged to discontinue these breeding experiments, and instead of resorting to pure strains obtained from outside sources we decided for several reasons to examine the questions of colour without reference to origin of animal.

Our preliminary investigations along these lines were directed towards obtaining information as to the most profitable colours to select, and to gain the required information a census of the colour of the 5000 animals present in the animal house was taken at intervals. The percentage of animals of each colour bearing tumours was recorded, due account being taken of the experimental conditions. Little information was, however, gathered by this means, which, in view of our later findings as regards difference in reaction to oils and tars, was not surprising.

In our early experiments we only made a note of the colour of animals which bore a tumour, and thus we have no data relating to colour of animals which died during the course of the experiments or were killed at its termination if they showed no evidence of tumour formation during the experiment. As we have the colour recorded of all animals which bore tumours, and which did or did not eventually bear malignant tumours, our malignant tumour data are consequently in some respects of more value than our benign tumour data. However, as almost all of our animals bore tumours when the tar experiments were terminated the absent data are few, but with oils it is another matter. In our recent experiments a record has been kept of the colour of every animal utilised, the records being of special value in considering the death-rate.

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Our animals were divided into 17 classes as regards colour, viz.:

(1) White.	
(2) Black.	(3) Black and white.
(4) Brown.	(5) Brown and white.
(6) Slate.	(7) Slate and white.
(8) Wild.	(9) Wild and white.
(10) Gold.	(11) Gold and white.
(12) Silver.	(13) Silver and white.
(14) Fawn.	(15) Fawn and white.
(16) Cream.	(17) Cream and white.

Slates included blues, greys, silver-greys and other related colours. In the case of mixed colours, other than white, the animal was classified according to the predominant colour. No. 1 and Nos. 12–17 were pink-eyed animals, the remainder having pigmented eyes. In this respect, however, there may have been some inaccuracies in our earliest records. Each animal was considered separately in respect to (*a*) the week in which a benign tumour arose, (*b*) the week in which the tumour became malignant, and (*c*) the week in which death occurred. The data (*a*), (*b*) and (*c*) were, of course, studied independently in the first place on separate sheets. The data available were utilised to find:

(1) The average week in which benign and malignant tumours respectively developed.

(2) The average benign-malignant interval.

(3) The deviation from the average week among colour classes.

(4) The average week in which death occurred in each group.

(5) The deviation from the average week of death among the colour classes.

(6) The percentage of animals bearing benign and malignant tumours respectively, alive at end of experiment.

Large sheets divided into inch squares, numbering 1–17 in a horizontal direction and 1′ to 10′ in a vertical direction, were used, there being a broad surrounding margin. The horizontal squares were for segregating colours, the experiments being arranged vertically. A square was allocated to each colour in a single experiment, the week in which a tumour arose on a particular coloured animal being entered in the appropriate square. The sum of the weeks in each square divided by the number of animals involved in each square gave us the average week for each colour in the experiment with which we were dealing. If we total horizontally we arrive at the average week for all the animals of the experiment, whence we derive the deviation from the mean of each colour class. A number of experiments being tabulated in this way we have only to total vertically to find the deviation of each colour or experiment from the mean. Below will be found the average week given by the different colour classes in 20 synthetic tar experiments, from which the deviation from the mean can be obtained. We note in passing that while pure whites gave nearly an average figure, white in combination with another colour was in all instances below the mean, and also always below the corresponding self-coloured animal's figure.

Colour	Average in weeks
White	14·228
Black	14·193
Black and white	14·031
Brown	14·545
Brown and white	13·181
Slate	15·107
Slate and white	14·103
Wild	14·520
Wild and white	12·333
Gold	15·452
Gold and white	13·666
Silver	15·061
Silver and white	13·125
Fawn	14·800
Fawn and white	13·400
Cream	14·200
Cream and white	12·333
Selfs (less white)	14·572
Piebalds	13·734
Average	14·291

The deviation from the mean in weeks calculated as above served only for the foundation of our colour-susceptibility analysis. We proceeded further because the carcinogenic potency of our oils and tars varied greatly in each group. Having obtained the average week of tumour arrival for each colour in a single experiment, we next converted these figures to a percentage basis, thus allowing us to mass together experiments with agents of variable potency. Our procedure will perhaps be most easily explained by giving an example. Thus in Exp. C 163 with a synthetic tar we found that the following was the average week in which the tumours arose in each class:

(1)	White	13·50			
(2)	Black	14·67	(3)	Black and white	11·62
(4)	Brown	14·62	(5)	Brown and white	14·75
(6)	Slate	13·80	(7)	Slate and white	13·00
(8)	Wild	20·00	(9)	Wild and white	—
(10)	Gold	13·33	(11)	Gold and white	—
(12)	Silver	15·33	(13)	Silver and white	9·00
(14)	Fawn	10·50	(15)	Fawn and white	—
(16)	Cream	19·00	(17)	Cream and white	17·00

To find what percentage of the whole was represented by each colour we total horizontally (not vertically, as above), which gives us 200·12 as the sum of the average weeks. As, however, only 14 out of the possible 17 colour classes were represented in the experiment our total must be multiplied by 17 and divided by 14, which gives us 243·00 as our dividend. The mean given by each experiment is thus always 1/17th of 100 (5·882 approx.). The percentages of 243·00 given by the different colours present are transferred to an ordinary double foolscap sheet, colours again being arranged horizontally and experiments vertically. In this case the percentages were:

(1)	White	5·556			
(2)	Black	6·038	(3)	Black and white	4·782
(4)	Brown	6·016	(5)	Brown and white	6·070
(6)	Slate	5·678	(7)	Slate and white	5·350
(8)	Wild	8·229	(9)	Wild and white	—
(10)	Gold	5·488	(11)	Gold and white	—
(12)	Silver	6·310	(13)	Silver and white	3·706
(14)	Fawn	4·320	(15)	Fawn and white	—
(16)	Cream	7·816	(17)	Cream and white	6·993

The deviation from 5·882 can now be found, which figures can in turn be stated in terms of deviation in weeks from the data already available. Ten such tar experiments are then massed by totalling vertically the percentages given by each colour, the average percentage of each colour among the ten experiments being found by dividing the totals by the number of experiments in which the particular colour occurred. In the present case the figures were as follows, the positions being given in brackets, with the most sensitive colour as No. 1:

White	5·800	(4)			
Black	5·994	(10)	Black and white	5·956	(8)
Brown	5·827	(5)	Brown and white	5·911	(6)
Slate	6·017	(13)	Slate and white	5·931	(7)
Wild	6·952	(17)	Wild and white	6·392	(16)
Gold	6·002	(12)	Gold and white	6·136	(15)
Silver	5·990	(9)	Silver and white	5·570	(2)
Fawn	4·785	(1)	Fawn and white	5·701	(3)
Cream	5·997	(11)	Cream and white	6·100	(14)

The groups of ten experiments are then further massed, the standard (bi-weekly applications) and daily experiments first being considered separately, and tars being kept apart from oils.

Colour sensitiveness was compared in the following manner:

(1) One colour class versus another colour class.

(2) One self-colour versus its piebald companion.

(3) One self-colour and its piebald companion versus another such combination.

(4) The sum of the self-colours versus the sum of the corresponding piebalds.

(5) The sum of the pigmented eyes versus the sum of the albino eyes.

(6) Absence versus presence of defective pigmentation in coat or eye (white factor).

We shall first consider benign tumours, and later analyse our malignant tumours and what we call the epithelioma lag. Our experiments were divided into four main groups:

(*a*) 40 experiments with petroleum oils painted daily.

(*b*) 50 experiments with shale oils painted daily.

(*c*) 30 experiments with synthetic tar painted bi-weekly.

(*d*) 30 experiments with synthetic tar painted daily.

The following was the number of animals dealt with in each of the colour classes:

Colour	Tumours: Petroleum	Shale	Tar, standard	Tar, daily	Total
White	283	510	337	317	1447
Black	147	323	198	323	991
Black and white	163	340	160	306	969
Brown	73	196	143	174	586
Brown and white	83	163	95	122	463
Slate	42	78	56	96	272
Slate and white	27	43	19	44	133
Wild	22	36	25	30	113
Wild and white	7	11	9	6	33
Gold	32	60	16	62	170
Gold and white	11	15	2	12	40
Silver	47	59	25	67	198
Silver and white	9	17	13	12	51
Fawn	18	27	34	57	136
Fawn and white	7	17	9	19	52
Cream	31	23	9	44	107
Cream and white	3	0	2	6	11
Totals	1005	1918	1152	1697	5772

The following are the results given by the different colour classes when painted with agents of each group. The position is also shown:

Colour	Petroleum oils		Shale oils		Total	
White	6·075	(10)	5·878	(8)	5·963	(9)
Black	5·652	(7)	6·176	(14)	5·945	(8)
Black and white	6·004	(9)	6·160	(13)	6·091	(11)
Brown	5·949	(8)	5·790	(7)	5·859	(7)
Brown and white	6·341	(13)	5·588	(5)	5·851	(6)
Slate	5·602	(5)	6·179	(15)	5·976	(10)
Slate and white	6·650	(15)	6·263	(16)	6·396	(17)
Wild	5·075	(1)	4·963	(1)	5·014	(1)
Wild and white	7·132	(17)	5·950	(11)	6·393	(16)
Gold	5·271	(3)	5·475	(2)	5·395	(2)
Gold and white	6·322	(12)	6·393	(17)	6·361	(15)
Silver	5·638	(6)	5·921	(10)	5·805	(5)
Silver and white	6·800	(16)	5·560	(4)	6·180	(13)
Fawn	5·178	(2)	5·969	(12)	5·647	(4)
Fawn and white	6·643	(14)	5·754	(6)	6·135	(12)
Cream	5·338	(4)	5·476	(6)	5·404	(3)
Cream and white	6·300	(11)	—		6·300	(14)

Colour	Tar, standard		Tar, daily		Total	
White	6·194	(16)	5·774	(6)	5·980	(13)
Black	6·026	(14)	5·828	(7)	5·925	(11)
Black and white	5·779	(7)	5·737	(5)	5·757	(4)
Brown	5·976	(12)	5·916	(9)	5·947	(12)
Brown and white	5·847	(10)	5·847	(8)	5·847	(8)
Slate	5·971	(11)	6·117	(16)	6·049	(16)
Slate and white	5·832	(9)	5·724	(3)	5·769	(5)
Wild	6·130	(15)	6·406	(17)	6·273	(17)
Wild and white	5·627	(6)	6·020	(14)	5·806	(7)
Gold	5·802	(8)	6·064	(15)	5·997	(14)
Gold and white	4·714	(1)	5·729	(4)	5·504	(1)
Silver	5·249	(2)	5·967	(12)	5·713	(3)
Silver and white	6·229	(17)	5·608	(1)	5·919	(10)
Fawn	5·470	(3)	5·611	(2)	5·554	(2)
Fawn and white	5·521	(4)	5·917	(10)	5·778	(6)
Cream	5·978	(13)	6·016	(13)	6·003	(15)
Cream and white	5·663	(5)	5·922	(11)	5·870	(9)

If we first scan our petroleum-oil results we cannot fail to see at once that our animals have not responded equally. That the presence of the white factor indicates a relative resistance to tumour formation is shown most clearly in this group, for not only is the piebald in every instance later in developing a tumour than its self companion, but the piebalds occupy the last eight positions out of the sixteen possible positions, whites being excluded. We note, however, that pure whites occupy an intermediate position, and also the curious fact that while wilds are the most sensitive of any animal wild-whites are the most resistant. As the average tumour arises in the petroleum group approximately in the 20th week of the experiment our figures represent a difference of about 7 weeks between wilds and wild-whites. The mean week for the arrival of tumours in the different groups, equivalent to 5·882 in our calculations, was:

Petroleum oil benign tumours	20·000
Shale oil benign tumours	15·258
Standard tar benign tumours	15·344
Daily tar benign tumours	14·291
Shale oil malignant tumours	27·236
Standard tar malignant tumours	21·242
Daily tar malignant tumours	20·701
Standard tar epithelioma lag	6·230
Daily tar epithelioma lag	6·800

so that to convert the deviation from 5·882 to weeks we have to multiply by:

3·4 in the case of petroleum oil benign tumours,
2·6 in the case of shale oil benign tumours,
2·5 in the case of all tar benign tumours,
4·6 in the case of shale oil malignant tumours,
3·6 in the case of all tar malignant tumours,
1·1 in the case of all tar epithelioma lag.

Thus, in the case of the petroleum oils where the wilds give a negative deviation of 807, and the wild-whites a positive deviation of 1250, making a difference of 2·507, we have:

$$\frac{20{\cdot}000}{5{\cdot}882} = 3{\cdot}4 \text{ approx. and } 2{\cdot}507 \times 3{\cdot}4 = 6{\cdot}994.$$

Thus, when painted with petroleum oils there was among our wild class and our wild-white class an average difference of 6·994 weeks in favour of wild sensitiveness.

Let us now consider for a moment our results when animals are painted with shale oil instead of petroleum oil. In this group 50 experiments were taken, the number of tumour animals concerned being 1918. Here there is evidently not the same discrepancy between the self-coloured animals and their piebald companions. The black, brown and slate couples have both members giving very similar figures, wilds and golds both being much more sensitive than the corresponding piebalds, while silvers and fawns (albino-eye animals) are apparently more resistant than the corresponding piebalds. Pure

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whites again occupy an intermediate position. Wilds are again the most sensitive, the gold-whites being in this case last. If the results with the 40 petroleum oils and the 50 shale oils are massed we find that in each colour couple, except brown, the self-colour is more sensitive than its piebald companion. The difference is most marked in the wild couple, next coming the gold couple. The least difference is in the brown couple, the self here being very slightly more resistant than its piebald companion.

Our third group consists of 30 experiments wherein the animals were painted twice a week with our synthetic tars. The number of tumour-bearing animals dealt with is here 1152. The remarkable fact emerges from an analysis of this group of experiments that colour sensitiveness seems to be the reverse to that found with the petroleum oils. In seven out of the eight couples the piebald animal is seen to be more sensitive than its self companion, the exception being fawn-white, an albino-eye animal. Wild, which has first position in both oil groups, here occupies last position. These results we felt could hardly be interpreted as due to chance. It is to be noted how the results with the golds especially support those given by the wilds. This is not surprising, as the majority of our animals classified as gold have a good element of the wild colour in them, as the wild have often a tinge of the gold. We have seen that golds when painted with petroleum oils and shale oils occupied third and second position respectively, while when painted with the synthetic tar they have the lowly position of fifteenth. Gold-whites occupied twelfth and last positions in the case of petroleum oil and shale oil respectively, while here they occupy fourth position. On the other hand the wild factor seemed to predominate the sensitive white factor in the wild-white class, so that the end result was a relatively resistant animal.

This reversal of sensitiveness as regards tars and oils was not anticipated, but at the same time we had at hand plenty of experimental results on different aspects of carcinogenesis to give us many ready explanations for this seeming paradox. However, before we gave the matter serious thought it was necessary to find out whether the difference between weak and strong agents, oils and tars, daily and bi-weekly painting, etc., accounted for the reversal. Accordingly we examined our fourth group of animals which consisted of 30 experiments with synthetic tar applied daily, as in the case of the oils. The number of tumour animals concerned in this group was 1697. The results in general terms agree with those of the previous group of tars. In six out of the eight couples the piebald animal is seen to be more sensitive than its self companion, the exception again being fawn, and in addition silver, both albino-eye classes. If we exclude whites and compare the position occupied by each colour class when painted with petroleum oils to that occupied when painted with synthetic tars, the two oil groups and two tar groups having been massed, we find that in 15 out of a possible 16 occasions the selfs occupy a higher position in the petroleum-oil experiments than they do in the tar experiments and the piebalds a higher position in the tar experiments than they do in the petroleum-oil experiments. The exception is pure silvers, an albino-eye class, which were sixth when painted with petroleum oils and third when painted with tars.

As the actual number of tumour animals in some of the colour classes is often none too large our results will perhaps be viewed with greater confidence if several classes are grouped together. We have excluded whites and compared the sum of the piebalds with the sum of their self companions in each of the four groups of experiments. Similarly, we have massed animals having no pigment in the eyes, including the whites, and compared them with the sum of those of which the eyes are pigmented. The following differences were obtained:

Agent	Self	Piebald	Difference	Pigmented eye	Albino eye	Difference
Petroleum oils	5·547	6·351	–804	5·877	5·890	– 13
Shale oils	5·836	5·952	–116	5·909	5·830	79
Tars, standard	5·870	5·796	74	5·900	5·843	57
Tars, daily	5·958	5·787	171	5·920	5·810	110

These massed results confirm as far as they can what has already been noted when considering the colour couples. We see that both as regards piebald and to a less extent albino eye there is a relative loss of resistance as the strength of the agent is increased, there being necessarily a reverse state of affairs among the selfs and the pigmented-eye groups. The inclusion of the pure whites in the albino-eye group partly accounts for the figures not running more nearly parallel with those of the piebald group; for the absence of pigment in the eye we presume to indicate the presence of the same factor as is concerned with the absence of pigment in the coat, etc. There are, however, many other factors besides absence of pigment to be taken into account.

If we compare the figures given by the animals having pigment with the remainder which have either albino eyes or are piebalds (white factor) the differences we obtain should be more striking. The following were the averages, blacks, browns, slates, wilds and golds constituting the wholly pigmented group and the remainder the white-factor group:

Pigmented self v. *white factor*.

Agent	Pigmented selfs	Deviation	White factor	Deviation	Difference
Petroleum oils	5·594	–288	6·072	190	478
Shale oils	5·834	– 48	5·925	43	91
Oils, total	5·734	–148	5·985	103	251
Tars, standard	5·996	114	5·716	–166	–280
Tars, daily	6·020	138	5·801	– 81	–219
Tars, total	6·009	127	5·765	–117	–244
Tars and oils	5·851	– 31	5·885	3	– 34

One could surmise fairly closely the differences which would result from this grouping of the animals from a survey of our previous figures. It is to be noted that there is not an equal number of animals on each side and that the deviation from 5·882 of the wholly pigmented is mostly greater than that of the pigment-defective animal. The reason why the tars and oils considered together do not bring the deviation on each side nearer to zero is in part at least due to the somewhat greater number of oil animals compared with the tar animals, viz. 2923 : 2849.

The deviation of each separate colour class was examined to ascertain that giving the minimum deviation. The brown class was found to have given the least deviation, and consequently this should be the colour of choice for experiments entailing relative potencies of carcinogenic agents.

Deviation from 5·882.

Colour	Petroleum oils	Shale oils	Tar, standard	Tar, daily
White	193	− 4	312	−108
Black	− 230	294	144	− 54
Black and white	122	278	− 103	−145
Brown	67	− 92	94	34
Brown and white	459	−294	− 35	− 35
Slate	− 280	297	89	235
Slate and white	768	381	− 50	−158
Wild	− 807	−919	248	524
Wild and white	1250	68	− 255	138
Gold	− 611	−407	− 80	182
Gold and white	440	511	−1168	−153
Silver	− 244	39	− 630	85
Silver and white	918	−322	347	−274
Fawn	− 704	87	− 412	−271
Fawn and white	761	−128	− 361	35
Cream	− 544	−406	96	134
Cream and white	418	—	− 219	40

In order to detect difference according to colour, other than white, we have not only considered each class of animal separately but we have taken a self-coloured animal and its piebald relative and compared the results given by this couple with other similar couples. Our 60 tar and 90 oil experiments analysed in this way give us the following figures, the position being bracketed:

Colour	Oils	Tars
White	5·963 (7)	5·980 (8)
Black—black and white	6·018 (8)	5·844 (3)
Brown—brown and white	5·855 (5)	5·902 (5)
Slate—slate and white	6·132 (9)	5·924 (6)
Wild—wild and white	5·447 (1)	6·138 (9)
Gold—gold and white	5·667 (3)	5·885 (4)
Silver—silver and white	5·914 (6)	5·788 (2)
Fawn—fawn and white	5·796 (4)	5·629 (1)
Cream—cream and white	5·500 (2)	5·976 (7)

As the average time for a tumour to develop in these tar experiments has been found to be approximately 15 weeks, it follows that if the above percentage figures give a true indication of relative sensitiveness, we shall expect a tumour to arise in an animal with a preponderance of fawn colour about $1\frac{1}{4}$ weeks earlier than in the case of a wild, when a synthetic tar is employed as carcinogenic agent. On the other hand, if a petroleum oil is used there will presumably be little difference whether fawn or wild colour predominates.

At first sight the difference in our figures may not be deemed very significant, but a consideration of our method for calculating the carcinogenic potency of our agents will at once make it clear that such a difference might

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materially affect the potency allocated to an agent. It must be understood that the records we have been analysing are of experiments mostly performed two or three years ago—experiments which were haphazard as regards tar or oil, date when commenced, source of animals, skill and observation powers of painter and recorder of results, etc.—so that many possible fallacies would appear to have been eliminated.

We have no definite evidence that pigment *per se* plays a part in sensitiveness, and we have only utilised colour as an indication of strain. Had we been in a position to utilise pure strains our examination into the question of strain susceptibility would no doubt have given more impressive results, for it is highly probable that many of our animals carry both dominant and recessive factors whereby they would react differently to a pure dominant or recessive, and consequently our results would be vitiated. The amount of error in our figures is not great, and from the practical point of view of no account. For instance, the average figure for all tar animals was 5·8816, the theoretical figure being of course 5·8824, as we dealt with 17 groups of animals. The error was introduced in the reading of the "Otis King," which we utilised in making some of our calculations.

Having made certain deductions from an examination of all tumour-bearing animals in our 150 experiments, we next sought to verify our conclusions by considering in a similar manner only tumours which became malignant. In this respect we studied chiefly the 60 tar experiments, there only being a yield of about 500 tumours which became malignant among the animals painted with oils, a number which we imagine to be too small to give us reliable figures. We have found that 1000 tumours seems to be the minimum number upon which one can rely for obtaining more or less consistent figures. Of the 2849 tar tumours 1669 became malignant, viz. 608 out of 1152 when the animals were painted bi-weekly, and 1061 out of 1697 when the animals were painted daily. The numbers in each colour class are given in the accompanying table:

Number of epitheliomas.

Colour	Tar, standard	Tar, daily	Total
White	208	206	414
Black	86	198	284
Black and white	92	198	290
Brown	59	109	168
Brown and white	43	78	121
Slate	26	51	77
Slate and white	8	25	33
Wild	20	16	36
Wild and white	9	3	12
Gold	10	36	46
Gold and white	2	8	10
Silver	12	42	54
Silver and white	5	10	15
Fawn	17	40	57
Fawn and white	5	16	21
Cream	4	20	24
Cream and white	2	5	7
Totals	608	1061	1669

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The results for malignant tumours given by each colour class in these 60 tar experiments were:

White	5·834	(6)			
Black	5·994	(16)	Black and white	5·879	(8)
Brown	5·937	(12)	Brown and white	5·978	(14)
Slate	5·977	(13)	Slate and white	5·800	(5)
Wild	6·031	(17)	Wild and white	5·979	(15)
Gold	5·856	(7)	Gold and white	5·907	(10)
Silver	5·921	(11)	Silver and white	5·476	(1)
Fawn	5·722	(4)	Fawn and white	5·680	(3)
Cream	5·881	(9)	Cream and white	5·494	(2)

The massed self-coloured animals gave 5·915 as against the piebalds' 5·845, a difference of 0·070 in favour of piebald sensitiveness. The pigmented eyes gave 5·935 as against the albino eyes 5·776, a difference of 0·159 in favour of albino-eye sensitiveness. If we couple the black with the black and white, etc., we obtain:

White	5·834	(4)
Black—black and white	5·937	(8)
Brown—brown and white	5·844	(5)
Slate—slate and white	5·907	(7)
Wild—wild and white	6·011	(9)
Gold—gold and white	5·865	(6)
Silver—silver and white	5·777	(2)
Fawn—fawn and white	5·710	(1)
Cream—cream and white	5·778	(3)

As was to be expected, if the epithelioma lag (see later) were normal our results should be similar, in a general way, to those which were given when considering benign tumours. It is to be noted that wilds are again last among the single groups and that the wild—wild and white couple is again last among the couples. The fawn and silver couples take first and second position respectively as they did when considering total tumours, while the cream couple jumping into third place enables the albino-eye animals to occupy the four first out of the possible nine places. We shall see that the epithelioma lag of the cream couple was relatively of very short duration.

It was a matter of interest to find what percentage of tumours in each class became malignant during the course of the experiment, without reference to date of becoming malignant. In this case the average time of survival and percentage of deaths of all tumour-bearing animals has to be taken into account, for a high percentage of deaths and a low survival time should lessen the possibilities for a high percentage of the tumours to undergo the malignant change. The table on p. 568 shows the percentages of tumours which became malignant, the percentage (benign and malignant) that died during the course of the experiments and the average duration of life (benign and malignant), given in weeks.

In the first place we note that the percentage of tumours which became malignant during the whole course of the experiment was in every couple less among the self than among the piebald. When the figures were massed we see that 6 per cent. less selfs than piebalds became malignant, or put in another way we shall expect among an equal number of animals painted for a similar period of time with the same tar only 9 self-coloured to become malignant for every 10 piebalds to become malignant. It may be pointed out also that the average duration of life of tumour-bearing animals was slightly longer among the selfs than among the piebalds, and that during the experiment there was 3 per cent. less deaths among the selfs. The longer life should of course operate in favour of the malignant change supervening, and yet in spite of this fact the selfs give the smaller percentage to show the change, an indication that the 9 to 10 ratio mentioned above is probably on the conservative side.

A consideration of pigmented eye versus albino eye shows in the massed results a relative albino-eye sensitiveness. The difference in the percentage of tumours to become malignant is 5·5, in the percentage of deaths during the experiment 7·6, and in the average life 0·201 weeks, all three differences pointing in the direction of an albino-eye relative sensitiveness.

Colour	% malignant	% of deaths	Average life in weeks
White	63·3	48·2	20·460
Black	54·5	43·7	20·548
Black and white	62·2	39·7	20·757
Brown	53·0	34·4	20·431
Brown and white	55·0	49·3	20·374
Slate	50·7	42·1	20·781
Slate and white	52·4	41·3	20·500
Wild	65·5	56·4	21·323
Wild and white	80·0	53·3	20·625
Gold	59·0	41·0	21·375
Gold and white	71·4	42·9	20·333
Silver	58·7	44·6	21·341
Silver and white	60·0	60·0	20·467
Fawn	62·6	47·2	20·326
Fawn and white	75·0	64·3	19·500
Cream	45·3	50·9	19·481
Cream and white	87·5	37·5	21·667
Selfs	54·9	41·0	20·632
Piebalds	60·9	44·0	20·552
Pigmented eyes	56·7	41·0	20·639
Albino eyes	62·2	48·6	20·440
Averages	58·6	43·5	20·565

Although we endeavour to make a microscopical examination of all skins suspected of being epitheliomatous, it sometimes happens that among animals dying during the course of the experiment this may not be possible. In such cases where the animals are unfortunately destroyed (by their companions, putrefaction, etc.), our diagnosis of malignancy rests upon clinical evidence. Such cases are a very small percentage of our totals, but in any event, it seemed of interest to find out the percentage of animals of each colour which were bearing malignant tumours when the animals were killed at the end of the experiments and were all subjected to a microscopical examination. It will be seen from the table given below that piebald and albino-eye sensitiveness to our tars is again indicated, although one out of the eight couples, viz. the gold—gold and white couple, should be reversed. Lastly, it may be well to draw attention to the seemingly high degree of sensitiveness of whites to the malignant influence of our tars. If due allowance is made for the possible error in the classes containing a small number of animals, it appears that whites may be the most sensitive class here.

Percentage of animals alive at end of experiments, bearing malignant tumours.

White	75·8		
Black	65·9	Black and white	67·6
Brown	64·9	Brown and white	71·8
Slate	60·2	Slate and white	67·2
Wild	82·5	Wild and white	100·0
Gold	67·4	Gold and white	62·5
Silver	78·4	Silver and white	100·0
Fawn	75·0	Fawn and white	90·0
Cream	53·8	Cream and white	100·0
Selfs	66·6	Piebalds	70·3
Pigmented eyes	66·9	Albino eyes	75·9
Average	69·6		

Another way of examining the question of susceptibility to the carcinogenic compounds in our agents is to consider what we call the epithelioma lag. The epithelioma lag is the interval required for a benign tumour to take on malignancy, and therefore is somewhat different to the estimation of malignancy according to the time it takes to supervene from the commencement of the experiment. We cannot of course find the epithelioma lag from the difference between the benign- and the malignant-tumour figures given already, because many of the benign-tumour animals die before malignancy supervenes and also because primary epitheliomas, *i.e.* those that do not develop on an animal already bearing a benign tumour, are not included in the epithelioma lag calculations.

The average epithelioma lag in weeks among 573 animals painted twice a week with synthetic tar was 6·230 weeks, while among 1027 animals painted daily with similar tars the average epithelioma lag was 6·800 weeks. The epithelioma lag of the colour classes among the 60 tar experiments calculated on the percentage basis as before was:

White	5·702	(9)			
Black	6·148	(12)	Black and white	6·277	(14)
Brown	5·778	(10)	Brown and white	6·313	(16)
Slate	5·657	(6)	Slate and white	5·688	(8)
Wild	6·190	(13)	Wild and white	6·436	(17)
Gold	5·683	(7)	Gold and white	6·292	(15)
Silver	5·492	(5)	Silver and white	5·456	(4)
Fawn	5·919	(11)	Fawn and white	4·789	(1)
Cream	5·157	(3)	Cream and white	4·882	(2)

On the whole it was expected that if benign tumours and malignant tumours gave similar deviations the epithelioma lag deviation would be zero. A correlation of our previous figures with those of the epithelioma lag will show, however, that this is not always the case, in some cases the sign being the reverse to that anticipated. The explanation of this may, however, be found by a consideration of the percentage of deaths and the average duration

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of life. If we couple as before to get an idea of the colours other than white we have:

White	5·702	(5)
Black—black and white	6·211	(8)
Brown—brown and white	6·107	(7)
Slate—slate and white	5·670	(4)
Wild—wild and white	6·272	(9)
Gold—gold and white	5·801	(6)
Silver—silver and white	5·480	(2)
Fawn—fawn and white	5·596	(3)
Cream—cream and white	5·084	(1)

Here we again get an indication of wild resistance and albino-eye sensitiveness. The position of the slate couple may be partly due to the possible inclusion of some silver animals in early experiments, classified as slates. For this reason it is probable that all our figures for slates should be a little higher than those actually given, and those for silvers similarly a little lower.

We had now reached a stage in our investigations when we were able to draw provisional conclusions. It appears that the white factor, indicated by an absence of pigment in the coat or eyes, implies that the particular animal concerned probably has a resistance above that of the average animal to the application of mineral oils, and below that of the average animal to applications of synthetic tars. The presence of pigment, equivalent to the absence of the white factor, necessarily implies the reverse state of affairs, the agouti (wild) being apparently the most sensitive of all colours to mineral oil applications, and the most resistant to synthetic tar applications. There are many explanations for these peculiar findings, such as difference in chemical constitution of oils and tars, difference in carcinogenic unit concentration, etc., but according to our conception the explanation of this reversal will be found in the domain of infection and immunity (tolerance).

As we had more material available we sought to verify our provisional conclusions by the examination of further experiments which brought the total number of tumours under consideration to nearly 10,000. We selected 90 additional experiments, viz. 40 in which the animals were painted with tars, and 50 in which they were painted with oils, the experiments being divided as before, into four sub-groups. The examination of these animals as regards colour sensitiveness to our carcinogenic agents in a general way amply verified the correctness of our previous conclusions. They also tended to verify other points about which we were not altogether happy, viz. the relatively high degree of sensitiveness of albino-eye animals, other than pure whites, to all agents, and especially that of fawns. Below is given a table of the deviation from 5·882 of the albino-eye, silver-fawn-cream couples (S.-F.-C. couples) in the eight benign-tumour and the four malignant-tumour groups, and it is to be noted that here there is not the difference between tars and oils, the deviation being negative (sensitive) in every instance. It will be seen also that the fawn couple considered alone gave in ten of the twelve groups a negative deviation, the shale oils applied daily giving a figure just above the mean in both the benign and malignant-tumour groups.

Agent	Duration of exp. in weeks	Tumours	Deviation from 5·882	
			S.-F.-C.	Fawn—Fn-Wt
Tars, daily	25	Benign	– 59	– 164
Tars, standard	25	,,	– 245	– 396
Tars, daily	40	,,	– 351	– 435
Tars, standard	40	,,	– 300	– 423
Shale, daily	35	,,	– 83	24
Shale, standard	45	,,	– 275	– 306
Petroleum, daily	50	,,	– 105	– 241
Petroleum, daily	60	,,	– 86	– 1479
Tars, daily	25	Epithelioma	– 97	– 75
Tars, standard	25	,,	– 218	– 350
Shale, daily	35	,,	– 399	12
Petroleum, daily	50–60	,,	– 292	– 903

The fawn factor appears to be a very sensitive factor, and if it has a greater all-over sensitiveness than the white factor fawns should give a lower average figure than fawn-whites. As a matter of fact the negative deviation of fawns was on an average – 342 and that of fawn-whites – 154, results quite in accordance with those expected. The position of pure whites is curious, and there are many other aspects of the subject which are somewhat ambiguous and which need further scrutinising. In the present paper we have limited ourselves to a discussion of the main aspects only, for a detailed discussion of the many problems opened up by our investigations would carry us much too far. It may be worth while, however, to mention two lines along which our studies have led us.

The relation of colour of animal to its sensitiveness to carcinogenic agents constituted only the preliminary part of our investigations. We are now examining pathological changes other than those of the skin, in a similar manner. We shall content ourselves here in mentioning only hyaline degeneration and thyroid enlargement (*J. Path. and Bact.* 1932, **35**, 219). In a selected series of experiments some 600 animals were examined for hyalinisation of the spleen, with the following results:

Agent	No. of animals examined		% with hyaline spleen	
	Pigmented eyes	Albino	Pigmented	Albino eye
Tars	181	78	34·4	62·8
Shale	69	40	48·1	60·0
Shale, treated	95	37	34·7	48·6
Petroleum	82	31	42·7	41·9

The figures indicate a very definite albino-eye sensitiveness on the part of the spleen itself or in some other way, except in the case of the animals painted with petroleum oils. It is interesting to note that more or less parallel results were obtained when thyroid enlargement was considered. Some 2250 animals, on being killed at the termination of selected experiments, were examined for marked thyroid enlargement, *i.e.* glands measuring over 3 mm. in their shorter diameter. Here the figures were:

Agent	Duration of exp. in weeks	No. of animals		% with very large thyroid	
		Pigment	Albino	Pigment	Albino
Tars	25	952	369	5·99	7·32
Tars	40	127	79	5·51	7·59
Shale oils	35	340	123	3·83	8·13
Petroleum oils	45	181	102	4·42	2·95

The percentage of very large thyroids given by the fawn couples was relatively very high, viz. 11·11, against only 5·32 given by the remainder of the animals at present under consideration. To appreciate the true significance of our figures the previous observations we have made on hyaline degeneration and thyroid enlargement must be taken into account. As regards thyroid enlargement it is specially to be noted that the animals did not die during the course of the experiments, so that all percentages are consequently lower than that of our animals as a whole. However, it appears that at the present time thyroid enlargement is not so persistent as formerly. While we are unable to discuss the various aspects of this subject here we feel that we should mention the important fact that in all four groups of animals the epitheliomatous had a greater percentage of very large thyroids than the non-epitheliomatous. The percentages of the four groups considered together were:

Epitheliomatous	6·84
Non-epitheliomatous	4·18

—in our opinion an interesting finding.

Summary.

An examination of some 10,000 tumour-bearing mice appertaining to 240 experiments comprising 24,000 animals has been made to find whether there was any difference in sensitiveness of the skin to our carcinogenic agents, according to colour of coat and eyes. Methods involving only elementary arithmetic have been utilised as we have, unfortunately, no statistical mind on our staff, and thus we have, presumably, failed to derive full benefit from the data available. We have ventured, however, to draw the following tentative conclusions as regards the particular animals under discussion, they being obtained from varied sources:

1. Self-coloured animals (excluding pure white) were more sensitive to petroleum oils (weak agents) than the corresponding piebald. (That is, a black was more sensitive than a black and white, etc.)

2. When tars (strong agents) were utilised instead of petroleum oils an opposite state of affairs existed, the self-coloured animal being more resistant than its piebald companion. This reversal may be explained on the grounds of cell tolerance.

3. Pink-eyed animals (excluding pure white) had a greater all-over sensitiveness of the skin to our agents than pigmented-eye animals.

4. On the whole the most sensitive animal was the pink-eyed fawn.

5. The frequency of hyaline degeneration of the spleen and marked thyroid enlargement was greater among pink-eyed animals, especially when fawn coloured, than among pigmented-eye animals.

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