THE RELATIVE POTENCY OF CARCINOGENIC TARS AND OILS.

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(*From the Laboratories of the Manchester Committee on Cancer.*)

(With 3 Graphs.)

In order to obtain, if possible, an accurate estimate of the relative potency of different carcinogenic agents when applied to the skin of mice, we have found it necessary to institute a numerical classification in addition to the drawing of graphs. The latter, although useful for demonstrating the difference in activity of two substances possessing more or less a similar degree of potency, are not altogether satisfactory for comparative purposes when the substances under consideration have widely different potencies. Thus, it often happens that an idea of the percentage increase or decrease in potency of an agent after chemical or physical treatment can be gained by consulting graphs but not nearly so rapidly nor so accurately as when potency figures, which as a matter of fact are calculated from the graphs, are available.

As stated in previous publications our graphs, which serve to demonstrate the essential details of the experimental results obtained from painting mice with different carcinogenic agents, are compiled from the percentage of living animals which bore or had borne tumours at each week of the experiment. A graph of the percentage of living animals which bore malignant tumours is also, in many cases, given, as well as a graph showing the death rate of the animals. Unless otherwise stated, 100 animals are used for each experiment, the applications being made twice a week with a camel-hair brush over an area of the back of approximately 5–10 mm. in diameter. Such a procedure constitutes what we call a standard experiment, and if no reference is made to variations from this procedure it may be assumed that the standard experiment has been adopted.

It has been mentioned that our potency figures are obtained from the graphs, and they are for the purpose of showing the carcinogenic activity of the agents tested. The figures are really derived from the average percentage number of living tumour-bearing animals for each week of the experiment, malignant tumours being considered separately. It was necessary to adopt some standard from which one could calculate the potency of a carcinogenic agent, but instead of utilising gas tar or some other agent it seemed better, for several reasons, to adopt some hypothetical substance as standard. Any large fluctuations in the graphs of the gas tar, or any variations in the susceptibility of the particular batch of animals used for the gas tar experiment were accordingly eliminated.

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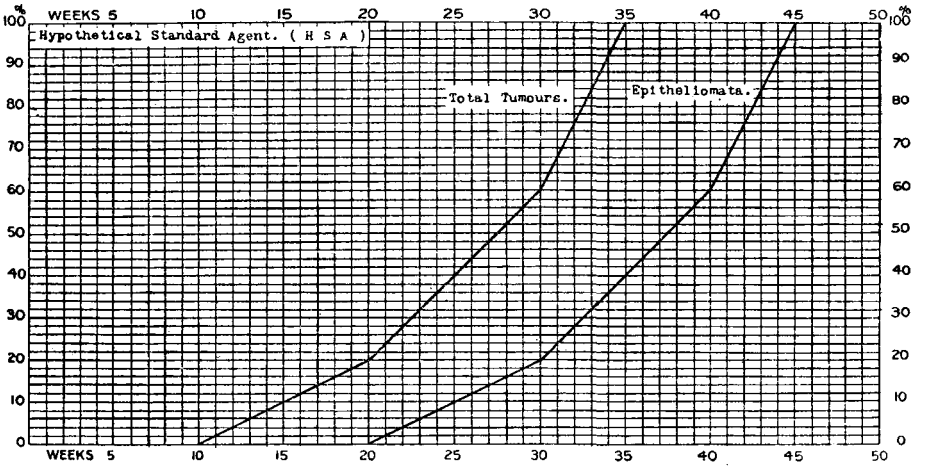
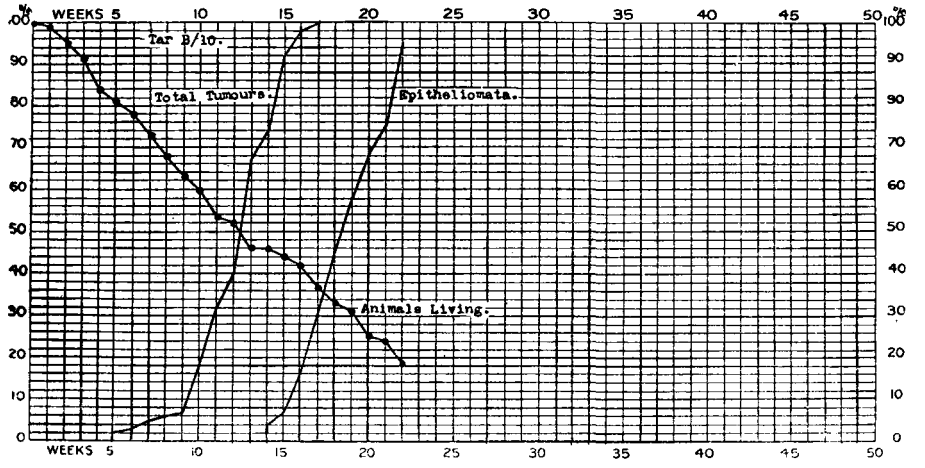
Our hypothetical standard agent, hereafter called the H.S.A., was arrived at by taking the mean figures of a large number of experiments with agents of varying degrees of carcinogenic activity. It is based upon results obtained with about 30,000 animals. The H.S.A. graph is shown in Graph 1. The first tumour is presumed to arise in the 11th week, while the most refractory



Graph 1.



Graph 2.

animal of the batch does not develop a tumour until the 35th week, at which time all living animals will thus be bearing or will have borne tumours. It will be noted that from the 11th to the 20th week the rise in tumour-bearing animals is 2 per cent. per week, during the next 10 weeks it is 4 per cent. and during the next 5 weeks it is 8 per cent. From the graph of the epitheliomata it will be seen that on an average it takes exactly 10 weeks for each tumour to change from benign to malignant, so that the first epithelioma does not make

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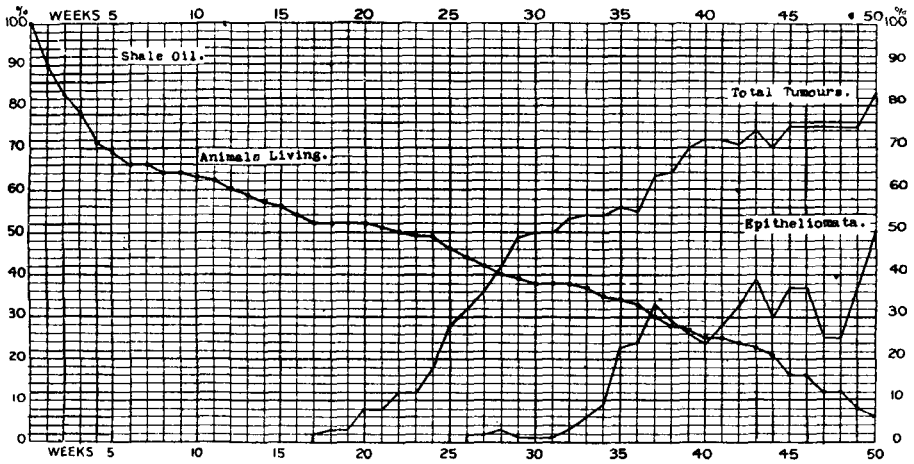
its appearance until the 21st week, and the last one not until the 45th week. By comparison we find that where the latent period between the commencement of the painting and the appearance of the first tumour is less than 10 weeks, the time taken for the malignant change to supervene is correspondingly shorter (see Graph 2), while with a weak agent, when the first tumour is late, it is correspondingly longer (see Graph 3). The stronger the agent the nearer do the tumour and the epithelioma graphs approach one another, and it seems that, very approximately, it requires twice the time for the production of a malignant tumour as it does for the production of a wart or papilloma, the multiplication factor becoming less as the agent becomes stronger, or the animal is more susceptible, and *vice versa.*



Graph 3.

Our potency figures, hereafter called the P. of an agent, are intended to convey an idea of the carcinogenic activity of the agent compared with that of the H.S.A. which has a P. of 100. Our figures are arrived at by the following method: The ratio of the mean percentage number of living tumour-bearing animals per week of the experiment to that of the H.S.A. during a similar period of time, added to the ratio of malignant tumour-bearing animals considered in the same way, multiplied by 100 and divided by 2 gives us the P. of the agent concerned. The P. of Tar *B*/10, of which the activity is shown in Graph 2, is 3802, while Graph 3 represents an agent with a P. of 48. In order to make matters quite clear the P. figures of Tar *B*/10 are herewith calculated.

The average percentage number of tumour-bearing animals for the first 17 weeks of the experiment is:

$$\text{Tar } B/10 \quad \ldots \quad \frac{544}{17}, \qquad \text{H.S.A.} \quad \ldots \quad \frac{56}{17};$$

the ratio being 9·71 to 1.

The average percentage number of living animals bearing malignant tumours during the whole course of the experiment is:

Tar $B/10$ ... $\frac{398}{22}$, H.S.A. ... $\frac{6}{22}$;

the ratio being 66·34 to 1.

Thus $(9{\cdot}71 + 66{\cdot}34) \times \frac{100}{2} = 3802$.

It will be seen that the time period from the date on which the last refractory animal developed a wart or an epithelioma, as the case may be, to the end of the experiment is not considered in making these calculations. When dealing with a weak agent the whole of the graph of the H.S.A. is utilised for comparing with part only of the graph of the agent concerned, while in the case of a strong agent only part of the H.S.A. graph can be utilised for comparing with the whole of the graph of the agent under consideration. Under certain circumstances the P. of an agent can only be arrived at by utilising the P. of an agent with an activity of somewhere near the same order, which has been already obtained from the figures of the H.S.A. This occurs when an agent is very weak or very strong, the graphs of which do not lie within the bounds of the H.S.A. graph.

As examples of the potency of several different agents we have:

Agent	P.
Hypothetical standard agent (Graph 1)	100
Pennsylvanian petroleum oil, No. 7	0·1
Californian petroleum oil, No. 9	3
Shale oil, No. 8 (Graph 3)	48
Pitch	25
Gas tar	94
Pinene synthetic tar	706
Concentrated synthetic tar (Graph 2)	3802
5% solution of highly concentrated synthetic tar	781

As a rule, for the quick appreciation of the percentage difference in the potency of two or more agents the P. figures are unsuitable. For this reason we more often consider the relative potency of an agent when comparing one agent with another. The R.P. of an agent is calculated directly, in the majority of cases, from the P. of the agent compared with the P. of some other agent, which latter is presumed to have an R.P. of 100. Thus as P. is the percentage potency of an agent compared with the H.S.A. so is R.P. the percentage potency of an agent compared with some other agent X. For example, when one wishes to have a clear conception of the relative amounts of carcinogenic substances in synthetic tars which have been made in a furnace tube under varying degrees of temperature, the difference in the activity of the resulting tars is very much more easily seen by consulting the R.P.'s than by consulting the P.'s. The potencies of six tars are given below in order to illustrate this point, a pinene synthetic tar made at 850° C. (Tar $B/1/850$) being taken as standard for the R.P.:

Tar	P.	R.P.
Tar *B*/1/500°	0	0
„ 1/600°	106	12
„ 1/750°	211	24
„ 1/850°	890	100
„ 1/950°	572	64
Tar *D*/1/850°	188	21

Again for comparing the action of chemicals on carcinogenic agents, or in any concentration or detoxication experiments, the R.P.'s give one an immediate idea of the percentage increase or decrease in activity of the agent under consideration. As examples:

Material	P.	R.P.
Oil No. 3	0·6	100
Distilled at 300–380/12 mm.	12	2000
Methyl sulphite extract	53	8833
Oil No. 8 (1)	48	100
Oxygenated at 150–160° C.	0·3	0·6
Oil No. 8 (2)	80	100
Oxygenated at 100° C.	52	65
Tar *B*/3	3745	100
Oxygenated at 100° C.	2369	63
Oxygenated at 150–160° C.	58	1

Thus oxygenation of a carcinogenic agent at 100° C. apparently destroyed or rendered inert 35–37 per cent. of the active carcinogenic substance while when the temperature was raised to 150–160° C. 99–99·4 per cent. was destroyed. These figures are of interest: (*a*) because they show that the percentage decrease in activity of two agents with widely different potencies was approximately similar under the same experimental conditions, and (*b*) because they indicate that the active substances present in shale oil (Oil No. 8) and in pinene synthetic tar (Tar *B*/3) are in all probability related chemically. It must be understood that these figures are only approximately correct, and as a rule one assumes that the further they are below those of the H.S.A. the greater is the possible error, because the total number of tumour-bearing animals becomes smaller as the agent becomes weaker, other things being equal. Naturally, the weaker the agent the smaller will be the number of animals surviving at the mean tumour development period.

While the potency figures are a measure of the power of a given agent to induce tumours they do not necessarily give us a true indication of the actual concentration of the carcinogenic material in the agent. This is because there is a definite concentration of the carcinogenic agent to which animals, on the whole, give the maximum relative response. This concentration we imagine to lie somewhere near five times that of the H.S.A., and we have adopted as a unit of carcinogenicity that quantity of carcinogenic material contained in 1/500th of a cubic centimetre of a hypothetical agent with a P. of 500. This unit of carcinogenicity, subsequently called the U.C. of an agent, is really a measure of the actual amount of carcinogenic material in a given quantity of any agent, or if carcinogenicity is a physical effect and not purely a chemical effect, it is a measure of an unknown physical state.

If it is assumed that the maximum relative response is obtained by utilising an agent with a U.C. of 500, then agents which have a U.C. above or below this figure will have to be diluted or concentrated respectively in order to get the best results. Numerous experiments have shown us that the U.C. cannot be calculated directly from P., and meanwhile we have not sufficient experimental data to justify the introduction of a constant from which we could base any calculations. As material accumulates it should not, however, be a difficult matter to arrive at a formula or at some method which will allow us to estimate with tolerable accuracy the concentration in carcinogenic units of any agent under consideration. The U.C. of an agent will rise or fall directly as it is concentrated or diluted, but P. will of course not vary in this way as the following examples show.

Oil No. 7, the Pennsylvanian petroleum oil mentioned as having a P. of 0·1, when concentrated by means of methyl sulphite extraction had a P. of 22; but the extract was 8 per cent. by volume of the whole oil, so that had the residual oil been completely deprived of active substances the extract should not have given a figure of more than 12·5 times that of the oil, instead of 220 times. Thus one is led to believe that although the P. of this particular agent is only 1/1000th that of the H.S.A., the difference in their U.C.'s is not nearly so great. Again if we consider the P. figures given by two concentrated synthetic tars and dilutions of the same we find that they do not correctly indicate the relative concentration in carcinogenic units of all five specimens. Thus:

Tar *B*/3 pure: P. = 3745, 10 per cent. P. = 176, 1 per cent. P. = 0·5.
Tar *B*/10 pure: P. = 3802, 10 per cent. P. = 750.

We see that the 10 per cent. solution of Tar *B*/3 figure has to be multiplied by 20 to approximate that of the pure tar, while that of the 1 per cent. solution has to be multiplied by approximately 7000. On the other hand, Tar *B*/10 in the pure state only had a P. five times that of a 10 per cent. dilution. One gathers from these figures that in order to obtain the maximum relative response with Tar *B*/3 a dilution of 20 per cent. should have been used, and with Tar *B*/10 a dilution of about 7·4 per cent. As a matter of fact Tar *B*/10 was really concentrated Tar *B*/3, and according to the carcinogenic activity of the residual tar left over after removal of Tar *B*/10 from Tar *B*/3 the activity of the former should be approximately three times that of the latter.

Summary.

A method has been devised whereby the potency of any given carcinogenic agent can be compared with that of a standard agent. This is called the standard carcinogenic potency of the agent (P.). The relative potency of the agent (R.P.), calculated from P., permits one to estimate the effect on the agent of different chemical treatments, dilutions, etc., the percentage increase

or decrease in activity of the agent being at once shown. A unit of carcinogenicity (U.C.) has been established, and a concentration of 500 carcinogenic units per cubic centimetre is considered to induce the maximum relative response on animals when the agent is applied twice a week. These standards have been devised as a natural sequence to the accumulation of a vast amount of experimental data the correlation of which necessitated some expedient more rapid and covering a wider field than the graphs formerly utilised.

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