THE RÔLE OF TOBACCO-SMOKING IN THE PRODUCTION OF CANCER.

(*From the University of Birmingham.*)

(With Plate II and 1 Text-figure.)

CONTENTS.

THIS investigation has been undertaken at the request of the Birmingham Committee of the British Empire Cancer Campaign. The chemical work has been carried out under the general advice of Prof. W. N. Haworth, F.R.S., in the Department of Chemistry, and the work on the clinical side under Prof. G. Haswell Wilson, M.D., in the Department of Pathology.

INTRODUCTION.

The well-established fact of the occurrence of a carcinogenic constituent in gas-works tar suggested the possibility of a causal connection between the formation of tar during tobacco-smoking and the development of cancer of the lip, tongue, or throat, apart from the effect of mechanical irritation due to hot and rough pipe stems.

Tar can accumulate in the pipe stem in considerable quantities during smoking, and it was thus considered possible that cancer might in certain cases be induced by the prolonged contact of the tobacco tar with the surface of the mouth and throat.

Kennaway (1924) showed that the carcinogenic substance of coal tar is not formed in any appreciable quantity below 550° C., and thus in an investigation on the production of tobacco tar it was of the utmost importance to consider and control the temperature factor with great care.

The investigation described in the present paper can be divided into the following sections:

(1) The determinations of the temperatures attained by different varieties of tobacco when burning in pipes. For this purpose both wooden and clay pipes were employed.

(2) The production on a large scale of a tar from the combustion of tobacco at temperatures and under conditions similar to those attained during ordinary

smoking. For this purpose a special apparatus was designed and is described in the following pages.

(3) The application of the tobacco tar to mice with the object of ascertaining whether a carcinogenic substance was present.

(4) The spectrographic examination of the tars.

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I. Determination of the temperature of burning tobacco in ordinary pipes.

As the carcinogenic power of coal tar is dependent on the temperature at which the tar is produced, it was evidently necessary first of all to determine the average temperature of tobacco burning in pipes under conditions of normal smoking, before preparing tobacco tar on a large scale artificially.

The temperature was determined by means of a thermocouple (at first a platinum-rhodium and finally copper-constantin and iron-constantin couples) attached to a millivoltmeter calibrated to read directly in centigrade degrees.

It was found that the temperature fluctuated at least 100° C. during the draw and puff, the maximum temperature occurring near the end of each draw.

Several types of tobacco were examined in both wooden and clay pipes, and the maximum temperatures observed are as follows:

Tobacco	Pipe	Temperature ° C.	Rate of smoking
A	Wood	535	Normal smoking
	Clay	590	Normal smoking
B	Clay	415	Normal smoking
		590	Hard smoking
C	Wood	370	Normal smoking
		480	Hard smoking
D	Clay	590	Normal smoking
		700	Hard smoking

The results show that in the case of tobacco smoked normally the temperature of combustion varied from about 370 to 590° C., according to the variety of tobacco employed.

With vigorous smoking, higher temperatures were observed, namely, from 480 to 700° C.

II. The production of tobacco tar on a large scale.

Having ascertained the average temperature at which tobacco burns in pipes, an apparatus was designed to produce tobacco tar in sufficient quantities for a long series of animal experiments.

By means of a thermocouple, it was possible to regulate the temperature of combustion, and it was arranged to produce tobacco tar at two different ranges of temperature: (i) 400–500° C., (ii) 700–800° C.

The apparatus was such that tobacco was allowed to burn in a series of

pipes with the aid of suction, and the smoke condensed under water at 37° C., thus producing accumulations of tar.

In addition, provision was also made for the inhaling suction to be intermittent, an exhaling suction coming alternately into action to simulate the puff-out of the smoker.

The general plan of the apparatus employed is shown in Fig. 1, see also Plate II. The method of working is as follows. The tobacco pipe *A* was packed with tobacco, and attached to tube *a*. The taps *T*, *U*, *V*, *W*, were then turned on and the glass taps, *R* and *S*, at the same time closed. As a result, a constant suction was applied to the pipe and it could be readily lighted. When the tobacco was alight taps *R* and *S* were subsequently opened.

The tap *U* had a filter pump attached to the flask *G*, and consequently the smoke was drawn through the water contained in the flasks *B*, *C* and *D*[1] (immersed in the thermostat *L* at 37° C.), and finally passed away through the flask *G*.

As the result of condensation in the collecting flasks *B*, *C* and *D*, tar gradually accumulated and was withdrawn from time to time for further treatment, described later on in this communication.

The inhaling suction induced by the pump at *U* was intermittent, a condition achieved by the following device, the object of which was to clear the condensation vessels from stagnant smoke at regular intervals and thus imitate natural smoking conditions, in which the accumulated smoke is removed by the puff.

The efficiency of this reversing or exhaling suction depends on the action of the *constant continual air-blow* introduced at a T-tube at *N*, the pressure of the blow being produced in the blower *F*. This blower consists essentially of a filter pump, the water and air from which pass into a large, strong glass vessel closed by a wired-on rubber bung. Through this bung also pass a very wide glass syphon to carry away the water delivered by the pump, and a glass tube provided with a glass tap which will deliver the "air-blow" to the apparatus. This tube is connected (via water trap *E*) to the coiled tube *M* by means of stout rubber tubing on which is placed a precision screw-clip *X*. The pressure of the air from this blower is capable of regulation to a value exactly equal and opposite to the suction applied at *K* (by altering tap *T* or the screw-clip).

Consequently a blast of air, preheated to 37° C. in the coil *M*, could be passed through the condensing flasks in the direction *D*, *C*, *B* without any pressure or suction being exerted on the surface of the water in *B* (or the air in tube *a*). In addition, when the suction applied to *G* is operative, the airblow augments this suction (instead of neutralising it).

The reason for not placing the end of tube *b* *under* the water in flask *C* now becomes apparent. If it were so placed, the flask would be emptied of

[1] Flask *D* in the early experiments contained animal charcoal as an adsorbent. Later the charcoal was replaced by water, as in the case of the other condensing flasks.

water when the exhaling suction from *K* started. (The object, successfully realised, of this flask is merely to increase the yield of tar.)

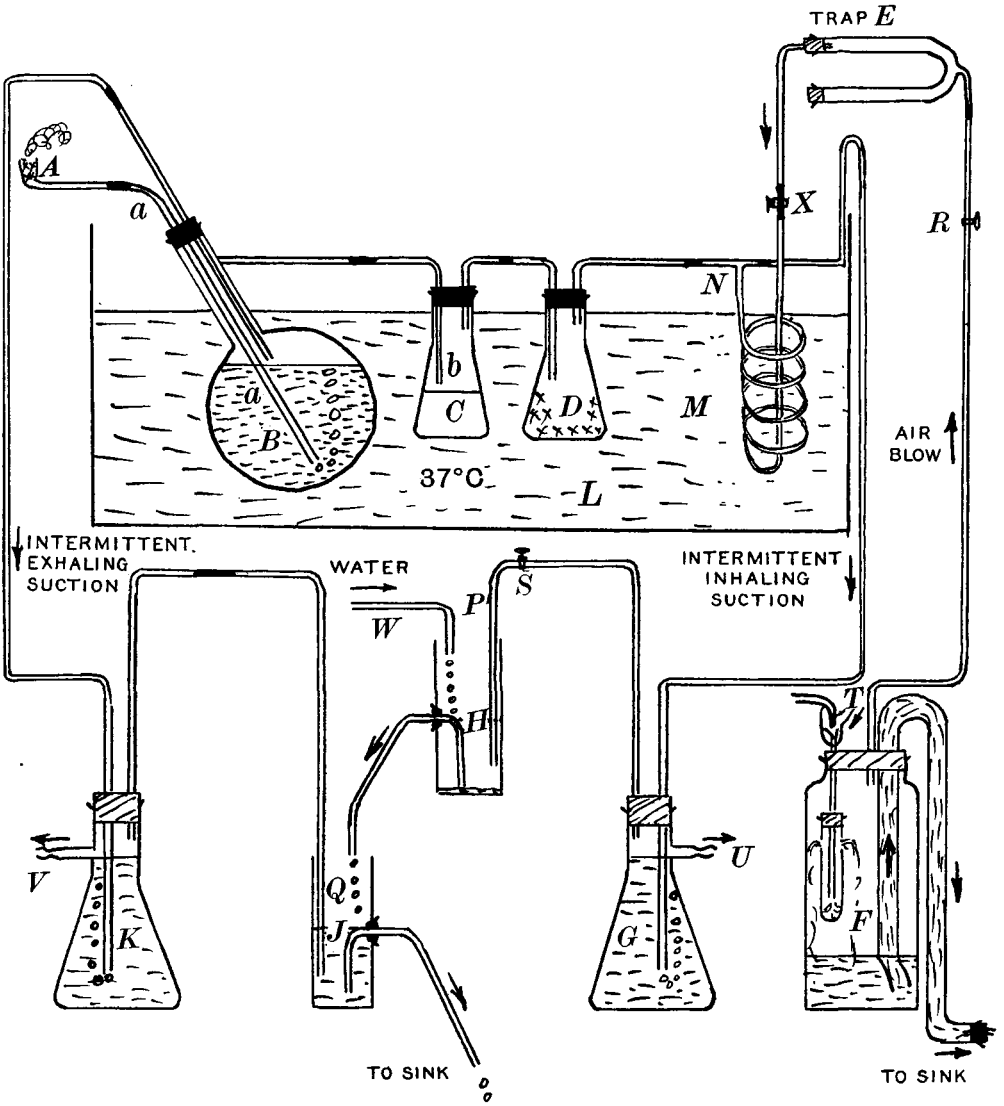

Fig. 1. Apparatus for smoking tobacco under regulated conditions.

The alternating device.

A constant stream of water, capable of regulation to any desired rate of flow, passes into the vessel *H*, and the surface of the water slowly rises and in time closes the end of tube *P*. The inhaling suction applied to valve *G* then becomes operative with respect to the pipe *A*, and continues during the time the level of water in *H* rises to the upper level of the syphon tube and syphons

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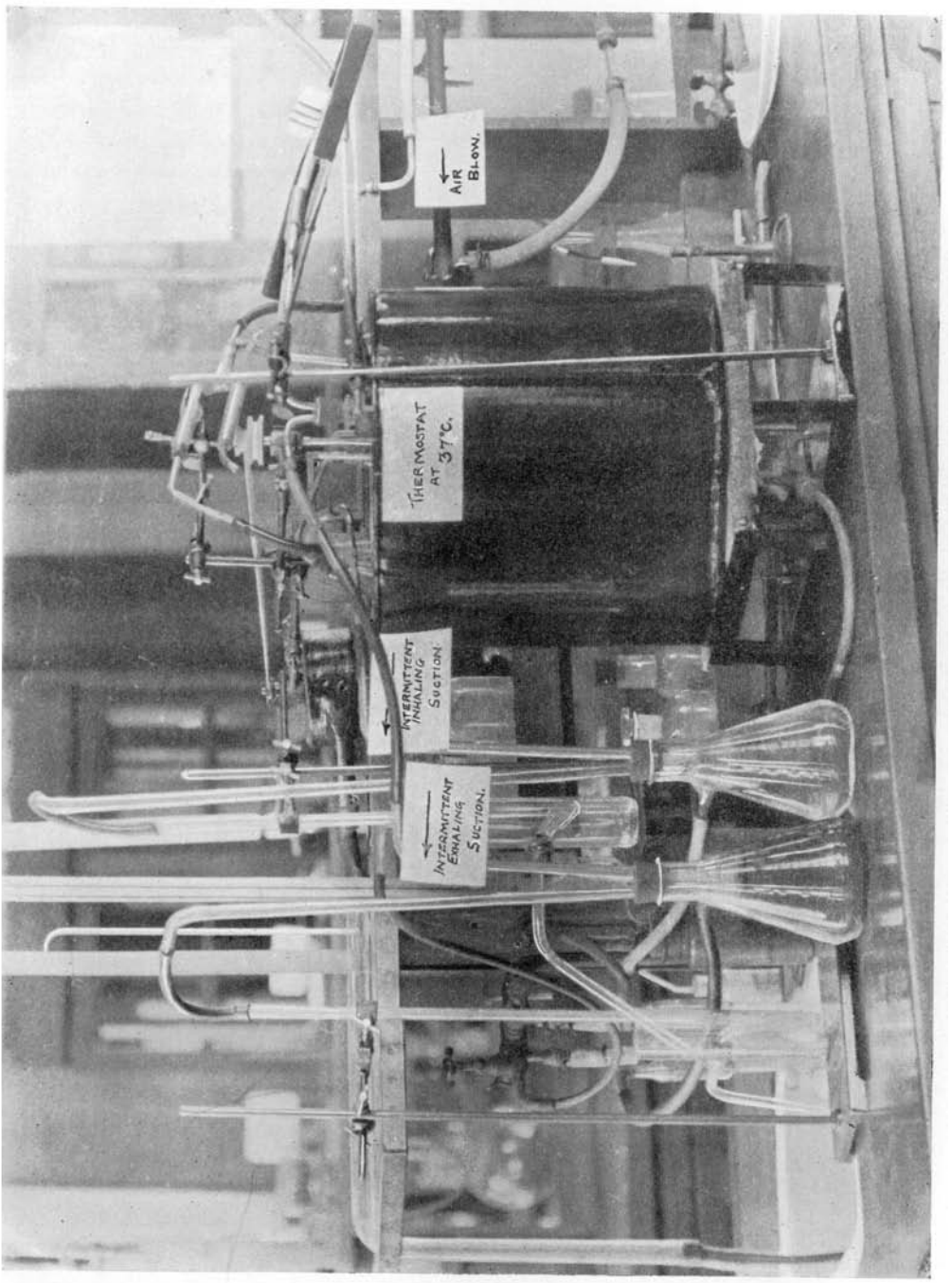

Apparatus for smoking tobacco from pipes.

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away. It ceases when P again becomes open to the air. The syphon conveys an equal volume of water into the vessel J; the end of tube Q becomes closed by water and the exhaling suction applied at K then starts to work through the apparatus. When the level of water rises to the top of the syphon, the water commences to syphon away to the sink, and as soon as Q is again open to the air the exhaling suction ceases, it being arranged (by careful regulation) that precisely at that moment P is again closed. Thus the alternating device continues quite automatically.

Two such pieces of apparatus were set up, one to smoke tobacco from wooden pipes, and the other from clay pipes.

At least four pipes were connected to the tube A by means of T-pieces in order to increase the production of tar.

The yield of tar varied with the type of tobacco, from 5 to 15 grm. being obtained from the combustion of 1 lb. of tobacco.

In order to remove water, the tar was subjected to distillation under reduced pressure at 45° C. The water was thus distilled off, and the tar remained in the distilling flask.

The crude tar was found to be toxic to mice, and with the object of diminishing the toxicity some of the tar was freed from bases and phenols by the following technique. A solution of the tar in chloroform was shaken with $N/1$ hydrochloric acid to extract basic substances. The separated chloroform layer was next shaken with $N/1$ sodium hydroxide in order to remove phenolic substances, then with distilled water until free from alkali, and finally dehydrated with magnesium sulphate. It was then evaporated under reduced pressure at 30° C. and the residual mass was the neutral tar fraction free from bases, acids and phenols.

The yields obtained in the course of several experiments are indicated below:

	Original tar	Neutral tar
	grm.	grm.
1	3·5	2·5
2	5·6	4·1
3	11·0	3·5
4	7·9	4·1
5	5·1	1·2

The percentage of neutral material removed by the two successive extractions was thus very inconstant, varying considerably with the type of tobacco used, and even varying from time to time with the same brand of tobacco.

III. By F. W. MASON LAMB, M.D.
AND (THE LATE) EDGAR SANDERS, M.Sc.

III. Animal experiments.

The tar preparations were applied to the skin at the back of the necks of the mice at intervals of about three days in alcoholic, benzene, or glycerol solution. The strengths of the solutions applied varied in different experiments, but as a rule were of the order of from 10 to 50 per cent. Some difficulty, as already mentioned, was experienced at the outset of the experimental work in consequence of the toxicity of certain of the tobacco tars, but this was got over (1) by employing solutions in the organic solvents already described, and (2) by using neutral fractions of the tars freed from toxic phenols and bases (animal experiment, series *c*).

Tobacco tar I. Produced at 400–500° *C.*

(*a*) 10 *per cent. alcoholic solution* (*wooden pipes*).

Mouse	1.	No obvious change in	17	months
,,	2.	,, ,,	16	,,
,,	3.	,, ,,	12	,,
,,	4.	Tumour developed in	16	,,

(*b*) 30 *per cent. solution in glycerol.*

Mouse	1.	No obvious change in	19	months
,,	2.	,, ,,	17	,,
,,	3.	,, ,,	14	,,
,,	4.	,, ,,	18	,,
,,	5.	,, ,,	19	,,
,,	6.	,, ,,	23	,,

(*c*) *Tar previously freed from phenols and bases* (*neutral fraction*).

(50 per cent. solution in benzene or 10 per cent. solution in glycerol.)

Mouse	1.	No obvious change in	17	months
,,	2.	,, ,,	16	,,
,,	3.	,, ,,	20	,,
,,	4.	,, ,,	16	,,
,,	5.	,, ,,	15	,,
,,	6.	,, ,,	15	,,
,,	7, 8.	,, ,,	22	,,
,,	9, 10.	,, ,,	14	,,
,,	11.	,, ,,	17	,,
,,	12.	,, ,,	16	,,
,,	13.	,, ,,	14	,,

(*d*) *Original tobacco tar dissolved in glycerol and applied hot* (*temperature* = 55° *C.*).

Mouse	1.	No obvious change in	6	months
,,	2.	,, ,,	6	,,
,,	3.	,, ,,	3	,,
,,	4.	,, ,,	12	,,
,,	5.	,, ,,	6	,,
,,	6.	,, ,,	10	,,

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Tobacco tar II. Produced at 700–800° *C.*

Alcoholic solution 25 *per cent.* (*wooden pipes*).

Mouse	1.	No obvious change in		6	months
,,	2.	,,	,,	4	,,
,,	3.	,,	,,	3	,,
,,	4.	,,	,,	3	,,
,,	5.	,,	,,	12	,,
,,	6.	,,	,,	12	,,
,,	7.	,,	,,	12	,,
,,	8.	,,	,,	9	,,
,,	9.	,,	,,	9	,,

Tobacco tar III. Produced at 400–500° *C.*

50 *per cent. alcoholic solution* (*clay pipes*).

Mouse	1.	No obvious change in		11	months
,,	2.	,,	,,	7	,,
,,	3.	,,	,,	9	,,
,,	4.	,,	,,	9	,,
,,	5.	,,	,,	12	,,
,,	6.	,,	,,	11	,,
,,	7.	,,	,,	12	,,
,,	8.	,,	,,	11	,,
,,	9.	,,	,,	11	,,
,,	10, 11, 12.	,,	,,	10	,,

The results show that out of the numerous mice painted with tobacco tar, only one animal developed a tumour, and this with a tar produced at 400–500° C. Further details concerning this growth are given in the following paragraphs.

Mouse 4. *Tobacco tar.* 10 *per cent. in alcohol* (400–500° C.).

First painted 4. iii. 29. Subsequently painted nine times, twice a week at intervals of 3 and 4 days.

Shaved, before tarring, 5. iv. 29. Subsequently painted nine times, twice a week.

Tar solution changed to 50 per cent., 3. vi. 29. Painting with new solution continued, at the usual intervals, sixty-six times, with little change in appearance of skin, apart from the thinning and reappearance of hair on the area tarred, until *one small wart* appeared, 11. iv. 30. Tarring continued for seventeen times more, at usual intervals, with progressive increase in the size of the wart, which was noticed to become much keratinised—like a small horn. Growth at the *base* was noticed on 1. vii. 30. The mouse was tarred twice more, and was found dead in the jar on 10. vii. 30.

Post-mortem examination, 11. vii. 30.

The internal organs were normal apart from some post-mortem changes, but the lungs showed extensive haemorrhage, and one or two small white cysts, similar to those found in other mice, and not metastases.

The growth of the skin itself showed at the edge a white tissue of firm consistence, poorly defined from the surrounding tissues; microscopic examination of this shows that it is an early epithelioma with much keratinisation,

but with definite downward penetration of the epithelium into the underlying tissues.

It is difficult to see why this mouse developed an epithelioma while its neighbours, treated in the same manner failed to do so. Both the shaving of the skin, and the change in the tar concentration occurred so early in the course of the whole experiment that they can scarcely have had much to do with it.

The fact that only one tumour has been obtained in the course of subjecting a large number of mice to the action of tobacco tar, compared with the very high incidence of cancer in mice treated with coal tar, indicates that tobacco is relatively unimportant in the causation of cancer.

IV. By E. L. HIRST, M.A., D.Sc.

IV. Report on the spectrographic examination of three samples of tobacco tar.

All the samples of tar whether obtained at low or high temperature exhibited fluorescence and the examination of the fluorescence spectrum given by them has now been completed. The conclusion is that in no case were there definite bands observed at the wave-lengths 4000, 4180, 4400 and consequently the samples do not possess the characteristics of many carcinogenic substances that are mentioned by Hieger in his original papers (1930). Instead of these bands the fluorescence spectrum was diffuse and apparently continuous for the whole range between 4000 and 4600. It appears then that the tobacco tars may contain carcinogenic materials combined with other substances which are also fluorescent and which confuse the spectrum and the result may be said to be inconclusive.

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