XXXIII. A NOTE ON THE BASES OF GASWORKS COAL-TAR WHICH ARE BELIEVED TO BE THE PREDISPOSING CAUSE OF PITCH CANCER, WITH SPECIAL REFERENCE TO THEIR ACTION ON LYMPHOCYTES, TOGETHER WITH A METHOD FOR THEIR INACTIVATION.

PART I. AUXETIC ACTION.

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It has long been known that gasworks coal-tar, the pitch derived from it and soot (which contains tar), cause chronic dermatitis, warts and ulcers leading to epithelioma, in the skins of the workmen employed at the briquette (patent fuel) works and tar distilleries. This specific predisposition to cancer brought about by these commodities has recently formed the subject of Home Office enquiries. The clinical evidence shows that it is only what is commonly known as coal-tar and the pitch derived from it by distillation that have this action; tar and pitch obtained from the blast-furnaces are harmless. The mechanical irritation caused by both varieties is identical, so that it is evident that some chemical factor is responsible for the predisposition to cancer above referred to, a fact borne out by the incidence of pitch cancer and warts caused by liquid petroleum [Legge, 1911; Lush, 1911; Ross, H. C., 1913, 1].

Although it is well known that gasworks tar differs widely in its chemical composition from blast-furnace tar, the latter being manufactured at a much lower temperature, it must also be remembered that the tar made at the gasworks and coke-ovens which is dangerous, is derived mostly from bituminous coal; the harmless blast-furnace tar, on the other hand, being made from the harder Scotch coal. [Ross, 1913, 2.]

H. C. Ross and J. W. Cropper [1910] put forward a working hypothesis connecting the onset of carcinoma and the predisposition to it with chemical substances called auxetics and kinetics. Auxetics are substances (most of them are amino-bodies) which induce cell-division in white blood cells, such as lymphocytes and other cells; kinetics are another group of substances (including most of the alkaloids) which excite amoeboid movements in cells.

The method of testing for both auxetics and kinetics is to mix the suspected solution with agar jelly, and to spread some blood cells on the surface of films prepared from this jelly. If auxetics are present in sufficient strength the lymphocytes after 10 minutes incubation at 37° will be seen to exhibit characteristic division figures; if kinetics are present, the cells (being examined without incubation) show exaggerated amoeboid movements. It is this test that has been employed in the following chemical experiments; the full details of the method have been described by Ross and Cropper [1911, 1]. In every case the test was repeated several times and throughout the experiments gave consistent results.

The present paper is concerned only with auxetic action, and the clinical bearing of this is not discussed; the question of the isolation of kinetics from tar will be described at a later date. In investigating the question of pitch cancer, Ross and Cropper [1911, 2] have detected auxetics and kinetics in gasworks tar and pitch, in soot and in liquid petroleum they were however unable to detect any kinetic whatever, and only a trace of auxetic in the harmless blast-furnace tar and pitch. A long series of experiments was made by these investigators and the subject is also dealt with in the Report of the second Home Office enquiry on pitch cancer [Lush, 1913].

From observations made by Ross and Cropper it was found that on fractionally distilling tar which had been found capable of yielding an aqueous extract active in inducing cell-division, the fraction distilling between 260°–320° comprised the whole of the active portion, that is to say the activity was associated with the anthracene fraction. Various pure substances known to occur in this fraction were tested by the above observers but found to be without action on lymphocytes. The following experiments were therefore undertaken to see whether the active agent or agents could be isolated.

EXPERIMENTAL.

Isolation of active basic fractions.

The tar used in the following experiments was a sample of the common variety which causes disease at the tar distilleries and briquette works and was kindly placed at our disposal by Mr Atkinson Butterfield.

Aqueous extracts were faintly acid in reaction.

Preliminary tests were made to ascertain the chemical nature of the substance or substances responsible for this auxetic action and in the first place an aqueous extract of the tar was used.

This was prepared by taking two volumes of water at 60° to 1 volume of tar, shaking for 1 hour, allowing to stand overnight and then centrifugalising or decanting off the aqueous layer. The washings prepared in this way were always faintly acid in reaction.

It was then found that on steam distilling with this original acid reaction, the substances capable of action on lymphocytes remained in the residue, but the kinetic action had apparently been destroyed or inhibited in some way as it was neither in the distillate nor residue. On steam distillation with a faintly alkaline reaction auxetic could be detected in both distillate and residue, that is to say the auxetic is slightly volatile in steam. The two steam distillations were then carried out consecutively on the same sample of washings and the result shown diagrammatically below, pointed to the active constituent being basic in character.

The active substance being therefore apparently of basic nature, acid extracts of the tar were made and it was found that this treatment yielded solutions which had a much stronger action on lymphocytes than had the aqueous ones. Acid extracts were accordingly used in all the following experiments, the acid employed consisting of two volumes of 5 per cent. hydrochloric acid to 1 of tar, and the extraction was carried out at 60° as in the previous case with water.

An attempt was then made actually to isolate the bases from the portion γ in the scheme above by means of chloroplatinic acid in the usual way, but after decomposing the platinum salt by means of sulphuretted hydrogen and then removing the excess of the latter by a current of air, no auxetic action could be detected. One explanation of this may be the adsorption of the active substance by the platinum sulphide which at first was formed in colloidal solution. A further explanation of this loss of activity was afterwards discovered and will be referred to later. The filtrate from the platinum salt was also found to be inactive after the removal of the platinum as above.

A further attempt was made using basic lead acetate as precipitant, decomposing the lead salt with sulphuric acid and then removing excess of acid by means of baryta. Here again no trace of auxetic action remained.

It then seemed necessary to obtain a larger and more concentrated supply of the active substance and methods with this end in view were next tried. It was found that on the addition of 10 per cent. caustic soda to an aqueous extract a precipitate was obtained which redissolved on continued addition of alkali and that this precipitate when filtered off and dissolved up in a little dilute acid (HCl) was a strong auxetic but did not keep well, rapidly losing its activity. In the same way the aqueous and acid extracts of tar likewise lose their activity on long standing in the air. With this method it was extremely troublesome to remove the excess of caustic soda, and if this is not done properly it is impossible to carry out the auxetic test with any hope of success as the alkali kills the cells, and an excess of salts even if neutral must be avoided¹.

The following method was then tried and found to be quite reliable. The tar was extracted as before with twice its volume of 5 per cent. hydrochloric acid, being shaken for one hour, and allowed to stand overnight and then centrifugalised. The supernatant liquor was precipitated with strong ammonia, care being taken not to add excess. A precipitate was obtained which on testing was found to be highly active while the supernatant liquor was inactive. The precipitate was collected, dried in a desiccator and again tested when dry and was still found to be highly active.

It is interesting to note that samples of alkaline condenser liquors and hydraulic main liquors from gasworks, which were kindly given by Dr Colman, were found after removal of sulphur to give the auxetic test. It is not certain whether these materials would be good sources of auxetics, as the latter deteriorate rapidly when exposed to the air in alkaline solution.

¹ The solutions must always be neutralised before testing on lymphocytes. Salts delay the diffusion of substances into cells so that they must not be in excess.

The precipitate from the ammonia may also be taken up in benzene and dried over anhydrous sodium sulphate, but before testing, the benzene must be very thoroughly blown off under a fan or distilled off under reduced pressure; the previous treatment is however to be preferred. In this way 85 g. of bases were extracted from about 1 cwt. of tar. These were distilled under reduced pressure and separated into five fractions.

Fraction	B.-P. at 16 mm.	Weight of distillate
I	0—100°	3.4 g.
II	100—150°	19.2
III	150—200°	18.2
IV	200—220°	8.5
V (Residue)	—	33

Fractions II and III were found to be highly active, I was extremely poor and IV and V were quite inactive.

II and III were then refractionated, but no great difference in activity could be detected in the fractions thus obtained. Both II and III oxidised rapidly in the air and in so doing lost their auxetic power, and this fact presumably accounts for the loss of action previously referred to in the weak aqueous and acid extracts of tar.

Further treatment of basic fractions.

Experiments were next made on the isolation of these active constituents and qualitative experiments showed the presence of nitrogen and possibly of sulphur.

An estimation kindly made by Dr Hartley of this Institute with van Slyke's apparatus showed that the nitrogen was not contained in any side chain but was bound up in the ring.

In this connection it was found that Kahlbaum's pseudocumidine, which is closely allied to the cumidine occurring in coal tar, was very feebly active. The test in this case requires great care as the conditions must be exactly right for any activity to be shown at all.

ψ -cumidine is easily benzoylated in the usual way yielding a white crystalline compound, Mp. 174–5° which has no longer any detectable action on lymphocytes. Attempts were then made to isolate individual bases from the mixture obtained by precipitation with ammonia, by means of benzoyl chloride, but little evidence of benzoylation could be obtained and no crystalline benzoyl compounds could be isolated. Had this been successful it would have been possible to separate the aniline bases from the pyridine bases and to ascertain whether auxetic action was an inherent property of either or both.

The next method tried was fractional crystallisation of the picrates. A saturated solution of picric acid was made, preferably in alcohol, and the basic fraction added drop by drop. A yellow solid at once separated out which crystallised readily from acetic acid. On fractional crystallisation two main fractions were obtained. The first more insoluble portion A, having a Mp. 199° – 201° , the second B (after several recrystallisations) Mp. 161° – 2° .

Analysis of A gave the following figures.

0.0895 g.; 0.1709 g. CO_2 ; 0.0219 g. H_2O .
 $\text{C}=52.11\%$, $\text{H}=2.71\%$.
 0.0999 g.; 0.1924 g. CO_2 ; 0.0252 g. H_2O .
 $\text{C}=52.52\%$, $\text{H}=2.80\%$.
 0.1188 g.; 16.8 cc. N_2 at 16° and 761.75 mm.
 $\text{N}=16.52\%$.
 0.1319 g.; 19.1 cc. N_2 at 16° and 760.5 mm.
 $\text{N}=16.80\%$.

The figures obtained for picrate A correspond with the following empirical formula, $\text{C}_{22}\text{H}_{14}\text{O}_9\text{N}_6$ which requires

$\text{C}=52.17\%$ $\text{H}=2.76\%$ $\text{N}=16.60\%$,

but nothing further is at present known with regard to the constitutional formula.

The quantity of picrate B, Mp. 161° – 2° , obtained was insufficient for analysis.

Experiments on the hydrolysis of picrate A were then undertaken with the object of isolating again and if possible identifying the active base from it, but the results so far have been somewhat unsatisfactory. In the first place the picrate was only hydrolysed with difficulty, and secondly it was found almost impossible to separate the very small amount of free base produced from the metallic picrates formed in the hydrolysis. Only when this was carried out with litharge was any free base obtained and the amount of this was much too small for identification to be possible.

The solution of this free base did however produce cell-division showing that it was one of the active constituents.

The hydrolyses were all carried out in air and possibly better results might be obtained by using an atmosphere of nitrogen, the active bases being very readily oxidised to inactive compounds.

Experiments on the inactivation of the auxetics of coal-tar.

In view of the theory that the susceptibility of pitch workers is due to the presence in the pitch used of the auxetics above described it seemed highly desirable to devise a method whereby these substances might be removed from the tar and pitch or at any rate inactivated. As has been

described above the auxetics can be almost completely removed from the tar by thorough washing with water or dilute acid. These methods however leave the tar in a state which renders its further distillation extremely troublesome and hence have practical objections.

A method has been recently patented by H. W. Robinson [1913] for the treatment of the tar or pitch by formaldehyde whereby the auxetics are inactivated, and an important test on a practical scale is now being made at the briquette works at Cardiff to see if this inactivation of auxetics prevents the disease occurring among the workmen.

The experiments described above, however, have suggested a simpler method of inactivation. It was noticed that aqueous extracts of tar rapidly lost their auxetic power if left exposed to the air for any considerable time. This was especially noticeable if the extracts had been made alkaline and hence the free bases unless dried or kept in an evacuated desiccator rapidly become inactive. The above facts suggested that the loss of activity was due simply to the oxidation of the auxetics and hence experiments were at once commenced with a view to rendering the tar innocuous by means of oxidation methods.

The following experiments illustrate the method finally adopted. 100 g. of tar were heated in a flask in an oil bath to 160° and a blast of air blown through. The tar was then extracted as before with acid, and the extract after neutralisation tested for auxetic action and found to be inactive in every case. A similar experiment was carried out with ozonised air and again the extract of the residue was inactive. The oxidation was quicker in this case than in the experiment where ordinary air was used. The auxetics present can therefore readily be rendered harmless by oxidation according to the method described and it is hoped shortly to try the process on a large scale. The experiments just described refer only to the auxetics, the inactivation of the kinetics being at present under investigation.

SUMMARY.

1. Methods are discussed for the separation of basic constituents of gas-works coal-tar which excite cell division.
2. The bases capable of exerting this action are found to occur in the anthracene fraction of the tar.
3. Two of these bases have been isolated as picrates but have not as yet been satisfactorily identified.
4. A method for the inactivation of these bases in tar is described.

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