LXVIII. THE DESTRUCTION OF 1:2:5:6-DIBENZANTHRACENE IN THE MOUSE.

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1:2:5:6-DIBENZANTHRACENE, injected into the breast muscles of a fowl, disappears from the site of injection in a few weeks [Chalmers, 1934; Berenblum and Kendal, 1934]. It seemed important to determine whether disappearance of dibenzanthracene was due to destruction, transportation to another part of the body or excretion unchanged. Preliminary evidence of destruction or excretion was forthcoming from an unsuccessful attempt to induce tumours of the reticulo-endothelial system of fowls: 20 fowls, which were given 30 weekly intravenous injections amounting to 20 mg. total dibenzanthracene (as colloidal aqueous solution), developed no tumours. Neither did they contain any dibenzanthracene in eight typical tissues when these were examined 2 years after commencement. Yet control experiments showed that dibenzanthracene was distributed at least to the heart, lungs and liver directly after intravenous injection (8 mg.).

Of course, proof of destruction of dibenzanthracene in the body actually requires an examination of the whole animal and its excreta for dibenzanthracene. Therefore, using mice as experimental animals, two methods were devised for quantitatively extracting the whole animal, obtaining an extract as free as possible from body pigments or fluorescent impurities and estimating dibenzanthracene spectrographically. The rate of disappearance of dibenzanthracene was then studied by estimating the amounts persisting in different mice at varying intervals after a standard intraperitoneal injection, while the question of excretion was studied by extracting the total excreta and examining such extracts for any traces of dibenzanthracene.

Experimental.

Preparation of extracts for spectrophotography (Method 1). The dead mouse was heated on a water-bath with 20 ml. 40% NaOH for a few hours. The sediment was separated and disintegrated with a little conc. HCl and the whole then neutralised with HCl and made alkaline with 5–10 g. anhydrous Na₂CO₃ (to prevent subsequent extraction of coloured material into the benzene). The mixture was evaporated to dryness on a water-bath and finally desiccated in a hot-air oven at 110° for several hours. The solid was finely powdered, excess of anhydrous Na₂SO₄ added and the whole extracted with boiling benzene either in a Soxhlet apparatus or by several changes of benzene under a reflux condenser. The extract was finally made up to a known volume (usually 100 ml.).

Numerous control tests with known quantities of dibenzanthracene gave reasonably consistent results. The amount of coloured impurities was very low, though it tended to vary somewhat. The chief objection to the method is the amount of trouble and time necessary to carry out a single extraction.

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Method 2. This is a simplified method which was used in later work (Table I) with a similar accuracy and efficiency. The mouse was boiled under a reflux condenser with alcoholic KOH (10 ml. saturated aqueous KOH and 80 ml. alcohol). 100 ml. benzene and 10–15 g. anhydrous CaCl_2 were added after cooling and the mixture was boiled for a further 10–15 min., then cooled and 8–9 ml. conc. HCl added. Most of the supernatant fluid was poured into a large separating funnel and shaken with several large quantities of dil. HCl to wash out all the alcohol (the acid being necessary to prevent the dibenzanthracene from passing into the watery phase as a colloidal solution). After a final washing with water it was shaken with several lots of dil. Na_2CO_3 solution (for the removal of pigments and other impurities) and filtered. A clear, almost colourless solution was obtained, representing all the dibenzanthracene from the mouse.

Estimation of the dibenzanthracene content of the extracts. The quantitative estimations of dibenzanthracene in mouse extracts were made by comparing the intensities of the characteristic fluorescent bands with those of a series of standard solutions of dibenzanthracene, photographed on the same plate. The apparatus employed was identical with that used by Hieger [1930] and others for the detection of carcinogenic compounds. The experimental error was less than 20 % while the limit of sensitivity was about one part of dibenzanthracene in 5–10 million of benzene, representing 0.01–0.02 mg. per mouse.

The disappearance of dibenzanthracene from the mouse. 2 mg. 1:2:5:6-dibenzanthracene dissolved in 0.5 ml. lard (previously filtered at 37°) were injected intraperitoneally into each of 44 mice. Two of the mice were killed and examined immediately and the remainder at intervals, after killing or death through natural causes, over a period of 160 days. A second group of 40 mice received intraperitoneal injections of a colloidal solution of dibenzanthracene in water. This solution contained about 16 mg./100 ml. and was injected in 6 doses of 2 ml. spread over a period of 4 days (representing about 1.9 mg. per mouse). Three mice were killed after the last injection and were found to contain 1.8 mg. each. Further estimations were carried out at intervals as in the previous group.

Fig. 1. 1:2:5:6-Dibenzanthracene detected in mice after intraperitoneal injection.

The results show clearly (Fig. 1) that dibenzanthracene disappeared from both groups of mice during the first 3 weeks. The disappearance was almost complete with the former group, whereas only about two-thirds disappeared with the latter. The following additional conclusions were reached from naked-eye and histological examinations of the animals: (1) Following the

injections of colloidal dibenzanthracene, small white nodules were found in the peritoneal cavity, usually attached to the viscera. These were present even at the end of the experiment and were found to be inflammatory nodules containing varying amounts of dibenzanthracene. Two such nodules in mice killed after 200 days contained 0.14 and 0.25 mg. of dibenzanthracene respectively. (2) In the group injected with the lard solution 1 of 9 mice surviving 20 weeks developed a tumour; in the group injected with colloidal solution in water, 4 of 9 developed tumours. These were all spindle-cell sarcomata arising in the peritoneal cavity and invading the surrounding tissues.

Other short experiments, with minor modifications in procedure, were carried out to confirm the above results. 12 mice received an intraperitoneal injection of 1 ml. of lard containing 1 mg. of dibenzanthracene (group A) and further 12 mice were given three daily intraperitoneal injections of colloidal dibenzanthracene, amounting to 1.2 mg. per mouse (group B). 5 mice from each group were killed immediately after injection (*i.e.* on the first day in group A and on the third day in group B) and the remainder were killed 12 days later. The mice were extracted (by method 2 described above) and the dibenzanthracene content was estimated in each.

Table I. *Dibenzanthracene detected in mice after intraperitoneal injections.*

Directly after injection		After 12 days	
Lard solution mg.	Colloidal solution mg.	Lard solution mg.	Colloidal solution mg.
1.05	1.2	0.39	0.46
1.0	1.1	0.44	0.33
0.95	0.8	0.25	0.72
1.0	0.9	0.44	0.12
0.9	1.0	0.33	0.50
—	—	0.46	0.50
—	—	0.52	0.40
Average 0.98	1.0	0.40	0.43

The results (Table I) confirm those of the previous experiment. Part of the dibenzanthracene injected had disappeared, the amounts being about the same in both groups, as was the case in the previous experiment on the 12th day.

The possibility of excretion of unchanged dibenzanthracene. Intraperitoneal injection of 1 ml. of lard containing 3.6 mg. dibenzanthracene was made into each of 5 mice. These mice were kept isolated in separate glass jars with a thick layer of loose asbestos fibre at the bottom of each jar. This, together with the accumulated excreta and food debris, was dried and extracted with benzene after 14 days, when the mice were killed and also extracted. In the 5 mice, 1.10, 1.00, 0.90, 1.25 and 0.95 mg. dibenzanthracene respectively were found, averaging 1.04 mg. per mouse. Thus about 2.6 mg. per mouse had disappeared. Yet no dibenzanthracene could be detected in the extracts of the excreta. Since the latter were rather coloured, the following control test was performed. Extracts of 14-day excreta from control mice were examined with known additions of dibenzanthracene. Detection of the compound in these mixtures was possible with quantities as small as 0.04 mg. Since therefore less than 0.04 mg. of the missing 2.6 mg. dibenzanthracene was present in the excreta in an unchanged form, dibenzanthracene must be metabolised in the body into a form no longer possessing the characteristic fluorescent bands.

DISCUSSION.

The power of the animal body to metabolise dibenzanthracene is of some interest. Dibenzanthracene is a stable aromatic hydrocarbon, insoluble in water and only sparingly soluble in a few organic solvents, whilst there is no existing evidence of enzymes in the body which might catalyse its destruction. However, the recent work on the relation between cholesterol, bile acids and oestrogenic compounds would suggest that the body is capable of more complex chemical reactions than have previously been considered.

The chief interest of the present findings lies in the fact that 1:2:5:6-dibenzanthracene is one of a group of related carcinogenic compounds. Its somewhat rapid destruction in the body therefore raises the question whether carcinogenic action is due to dibenzanthracene itself or to products of its decomposition. In the above experiments, more tumours were developed when an appreciable amount of dibenzanthracene persisted in the body for many months (*i.e.* following injections of the colloidal solution) than when only traces of the compound remained after the first 3 weeks (following injections in lard). This suggests that dibenzanthracene is the carcinogenic agent but no conclusion based on such slight evidence merits any emphasis. The persistence of dibenzanthracene in the body when injected in colloidal solution is probably due to aggregation into nodules in the peritoneal cavity with consequent encapsulation.

It has been suggested that carcinogenic compounds appear in the body through some disturbance in sterol metabolism [Kennaway and Cook, 1932]. If however these compounds are destroyed in the body as readily as dibenzanthracene, they will have to appear with corresponding rapidity, if experimental evidence for this suggestion is ever to be obtained.

SUMMARY.

1. A method for the quantitative estimation of 1:2:5:6-dibenzanthracene in the mouse is described.

2. Estimation of dibenzanthracene at different times after intraperitoneal injection (2 mg.) showed that the greater part disappeared from the animal. The disappearance was more complete when the dibenzanthracene was injected dissolved in lard than when it was injected in the form of a colloidal solution in water. No dibenzanthracene could be detected in the excreta of such animals.

3. It is therefore concluded that the mouse is able to metabolise dibenzanthracene.

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