CLXVII. THE ROLE OF 1:2:5:6-DIBENZANTHRACENE IN THE PRODUCTION OF FOWL TUMOURS.

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THE injection of 1:2:5:6-dibenzanthracene dissolved or suspended in fatty media into the breast muscle of fowls is followed, after a variable latent period, by the production of sarcoma in a significant number of birds [Burrows, 1933; Peacock, 1933]. Such tumours fulfil the accepted criteria of malignancy, in that they invade the normal tissues of the host and metastasise freely, giving rise to secondary tumours of similar histology. They can be transplanted in homologous series but so far have not been transmitted successfully by filtrates or desiccates.

The importance of determining the action of dibenzanthracene¹ in the production of these growths has been realised. Changes in rabbit serum-esterase values following intraperitoneal injection of a dibenzanthracene solution have been investigated [Green, 1934]. More recently it has been suggested that dibenzanthracene is altered in the body to form a water-soluble compound before it can produce biological effects [Boyland and Boyland, 1934].

The injection of a solution of dibenzanthracene in fat into the breast muscle of fowls might be followed by:

- (1) Localisation of the dibenzanthracene in the muscle, or deposition in connective tissue or phagocytic cells.
- (2) Active or passive removal from the site of injection.
- (3) Chemical change.

The consideration of these possibilities involved an estimation of the quantity of dibenzanthracene present in the breast muscle of fowls at predetermined intervals after injection. No work appears to have been published on this aspect of the problem. A method for detecting dibenzanthracene in small quantities was necessary, since it was decided to inject amounts similar to those employed in producing experimental tumours. As a solution of 0.0005 % dibenzanthracene gave a well-defined absorption spectrum, the spectrographic method was used for its detection.

EXPERIMENTAL.

The absorption spectrum of dibenzanthracene has been described by Clar [1929] who examined a benzene solution contained in an adjustable cell. In the present investigation, standard Hilger cells, which hold 5 mm. layers of liquid, were used for the spectrum analysis in conjunction with a Hilger Sector Photometer and Spectrograph (E, 3)². A condensed iron spark was used as the source ultraviolet radiation. We are indebted to Dr Cook of the Cancer Hospital, London, for supplying a pure specimen of dibenzanthracene, which had been proved to be

¹ In every instance "dibenzanthracene" denotes "1:2:5:6-dibenzanthracene."

² The property of The British Empire Cancer Campaign.

carcinogenic by the London workers and which gave rise to fowl tumours in the experiments referred to previously. The dibenzanthracene absorption curve obtained corresponds closely to that given by Clar, and the differences observed are probably due to changes in technique. Fig. 1 shows the absorption curve over the range which is of special interest in this work (2600–3100 Å.).

Fig. 1.

Dibenzanthracene (0.0005 %) in chloroform: extinction coefficient 2.8, maximum 2995–3000 Å.

Dibenzanthracene (0.0005 %) in alcohol: extinction coefficient 2.8, maximum 2965–2970 Å.

Extinction coefficient = $\frac{\log I_0/I}{d}$ where I_0 is the intensity of incident light, and I the intensity of emergent light after passing through d cm. of liquid.

The absorption curve of dibenzanthracene in chloroform when compared with the absorption curve given by Clar shows:

- (1) A similar band with maximum at 3000 Å.
- (2) An indication of a shallow band instead of a well-defined one at 2900 Å.
- (3) No rapid increase in absorption below 2750 Å.

Thus, the chief difference occurs at wave-lengths less than 2750 Å.

It should be noted, however, that benzene, the solvent used by Clar, shows general absorption in this region.

Preparation of extracts.

In preparing a chloroform extract of an injected breast muscle, the same procedure was followed in all cases. The bird was killed and frozen to prevent loss of liquid fat, and the breast, together with the sternum, was removed; then the injected breast was excised, ground up with sand and extracted with chloroform in a Soxhlet apparatus for 6 hours.

Preliminary experiments.

Six fowls, injected with a solution of dibenzanthracene in egg-yolk fat or in lard with the object of producing tumours, which had died without showing any evidence of malignant change, were examined. The time from injection until

death varied from 18 months to 2 years. Where cysts or nodules had formed at the site of injection, these were examined separately, but otherwise the whole breast was extracted. None of the extracts, although concentrated to the point of general absorption in the ultraviolet, showed selective absorption. As this seemed to indicate that the dibenzanthracene had been removed from the site of injection or had changed into some other compound, it was decided to examine fowls killed shortly after injection.

Series I. Dibenzanthracene in chicken fat.

Four Plymouth Rock fowls (9 months old) were injected in the breast muscle with 5 ml. of a 0.10 % solution of dibenzanthracene in chicken fat.

No. of fowl	Time from injection until death	Concentration of extract examined	Total dibenzanthracene in breast muscle at death (mg.)
716	Killed immediately	1000	5.0
700	1 day	1000	3.5*
665	5 days	1000	1.8*
724	21 days	1000 > 250	Not detected

* These approximate values are taken from the graph (Fig. 2), which shows the absorption spectra of breast muscle extracts from fowls 716, 700 and 665. A 0.5 % solution of chicken fat in chloroform did not exhibit any absorption in the ultraviolet.

Fig. 2.

Series II. Dibenzanthracene in egg-yolk fat.

Seven Rhode Island Red chicks (6 weeks old) were injected in the breast muscle with 0.5 ml. of a 0.20 % solution of dibenzanthracene in egg-yolk fat.

The livers of chicks 1003, 1004 and 1007 were extracted with chloroform by a method similar to that adopted for the treatment of breast muscles. The extracts were examined spectrographically, but no dibenzanthracene could be detected.

The total chick excreta were collected during the course of the second series of experiments. A chloroform extract of these excreta, however, showed intense

No. of chick	Time from injection until death	Concentration of extract examined	Total dibenzanthracene in breast muscle at death (mg.)
1001	Killed immediately	200	1.0
1002	1 day	200	0.6*
1003	2 days	200	0.5*
1004	3 days	200	0.4*
1005	4 days	200 > 50	< 0.1†
1006	7 days	200 > 50	< 0.1†
1007	14 days	200 > 50	< 0.1†

* These approximate values are taken from the graph (Fig. 3), which shows the absorption spectra of breast muscle extracts from chicks 1001, 1002, 1003 and 1004; and the slight general absorption of 0.25 % egg-yolk fat in chloroform. The absorption spectrum of a 400 ml. extract from chick 1001 (i.e. a solution containing 0.5 mg. dibenzanthracene per 200 ml.) is inserted for purposes of comparison.

† To demonstrate that 0.1 mg. dibenzanthracene could be detected, 0.5 ml. egg-yolk fat solution containing 0.02 % dibenzanthracene was added to the breast muscle extract from chick 1005, which had not shown selective absorption. When re-examined with the spectrograph, the dibenzanthracene spectrum was observed.

Fig. 3.

absorption in the ultraviolet, and it could not be tested until compounds which would mask the dibenzanthracene spectrum had been removed. Further work on this aspect of the problem is proceeding.

DISCUSSION.

The amount of dibenzanthracene present in the breast muscle of fowls injected with fatty solutions of this substance falls off rapidly from the time of injection, and after a few days decreases to less than one-tenth of the original quantity. This observation holds for all fowls examined, and it is worthy of note that the time taken for the dibenzanthracene to disappear is short compared with that required for clinical or histological evidence of a malignant change. It is evident that the bulk of the injected dibenzanthracene is transferred or under-

goes chemical change, though it is possible that minute quantities are phagocytosed and remain localised in this way.

No sign of dibenzanthracene has been found in the livers of any of the birds examined. The possibility of detecting dibenzanthracene in chick excreta is now under consideration. If dibenzanthracene is not found in the excreta the possibility of localisation in parts of the body other than the liver must be considered before any assumption of chemical change can be made.

SUMMARY.

1. The absorption spectrum of dibenzanthracene is described.
2. The quantity of dibenzanthracene present in the breast of the fowl, at short intervals after injection, has been determined and represented graphically to show that the dibenzanthracene rapidly disappears from the site of injection.
3. The possibility of the transference of this compound to the liver has been investigated, but its presence there has not been detected.
4. Examination of excreta to detect the possible elimination of dibenzanthracene as such has been attempted, but the tests applied have not proved satisfactory.

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