Mesoblastic Tumours following Intraperitoneal Injections of 1:2:5:6-Dibenzanthracene in a Fatty Medium.

By Harold Burrows.

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(From the Research Institute of The Cancer Hospital (Free), London.)

[Introduction.

Attention was first drawn to 1:2:5:6-dibenzanthracene as a carcinogenic substance during an inquiry into the fluorescent spectra of coal tars carried out by W. V. Mayneord in collaboration with E. L. Kennaway at this Institute in 1927 and later continued by I. Hieger (1930). Since then the substance has been prepared in a very pure form by J. W. Cook (1931, a), who has made some closely allied compounds which are also carcinogenic (1931, b, c; 1932). Kennaway (1930) and Hieger (1930) have already recorded the causation of epitheliomata by 1:2:5:6-dibenzanthracene dissolved in benzene applied to the skin of mice; and Burrows, Hieger and Kennaway (1932) have described the production of connective tissue tumours in rats and mice by injecting 1:2:5:6-dibenzanthracene dissolved in lard into the subcutaneous tissues. These tumours conformed to the usually accepted criteria of malignancy; grafted strains have now reached the 23rd and 36th generation in mice and the 18th and 20th generation in rats.

Technique.

The present paper sets out some results of the intraperitoneal injection of fatty solutions of 1:2:5:6-dibenzanthracene. This substance was dissolved in a concentration of 0.4 per cent. in olive oil by heating at temperatures not exceeding 100° and the solution was then emulsified in 3 volumes of 5 per cent. gum acacia in water usually made alkaline to pH 8.2 in order to assist emulsification. The concentration of the hydrocarbon in the emulsion was thus 0.1 per cent.*

Intraperitoneal injections of this emulsion were made once a week into the right hypogastrium of rats and mice. At the commencement of the experi-

* The preparation of this emulsion was kindly undertaken by the Crookes Laboratories (British Colloids, Ltd.), to whom we are greatly indebted.
ments the rats received 0.5 c.c. and the mice 0.05 c.c., these doses being soon raised to 1 c.c. and 0.1 c.c. respectively.

The original purpose of this experiment was to cause the formation of a tumour in a part of the animal's body remote from the site of the injection of dibenzanthracene. It was thought that if this substance entered the general blood stream it might become subsequently localised and concentrated in an area of focal inflammation artificially produced, and so bring about a new growth in the inflamed tissue. Accordingly various substances (e.g., starch, peptone broth) were injected into the napes of these rats and mice to set up an aseptic inflammation in this region. In no instance has a tumour occurred in the inflamed nape, but several of the animals have already succumbed to peritoneal tumours.

The account which follows is concerned mainly with a group of 10 rats which had been given weekly intraperitoneal injections of the emulsion of 1:2:5:6-dibenzanthracene described above, 2 c.c. of peptone broth being introduced into the nape of the neck on the same occasions, fig. 1.

**Results.**

The administrations were commenced on May 22, 1931, and the last rat was killed in the 49th week of the experiment. Eight of these 10 rats developed definite peritoneal tumours. Of the remaining two, one, rat No. 2, was killed in the 26th week with a large liver and ascites, and showed a condition of peritoneal thickening which suggested that tumour formation might have occurred later had the animal lived long enough. The remaining rat, No. 5, died and was eaten by its fellows, so that no post-mortem examination was possible.

The average life of the eight tumour-bearing rats after the commencement of the experiment was 40 weeks, the shortest life being 23 weeks, during which period 21 injections were given, and the longest life 49 weeks. The total amount of 1:2:5:6-dibenzanthracene administered in the former case was approximately 20 mg. and in the latter 43 mg.

The accompanying chart shows the history of these animals in graphic form (fig. 1).

The records of this group of rats are briefly as follows:

**Rat 1.**—Killed in the 23rd week of the experiment. The rat at this time appeared ill and an abdominal tumour could be recognised during life. P.M. examination showed a rounded mass in which the cæcum, intestines and mesentery were inextricably bound. The mass, which was freely movable within the limits allowed by the mesentery, had no adventitious attachments to the abdominal
wall or to the liver, stomach or other viscera. No other tumours were observed. Microscopical examination showed the tumour to consist of polymorphic cellular tissue in which fusiform and oval cells predominated. Some giant cells were present. In one place the growth had invaded the intestine, and destroyed the muscular coats.

Fig. 1.—The numbers 1–10 below the base line are the reference numbers of the rats. Each vertical line represents the duration in weeks of the life of one rat from the commencement of the experiment. T = peritoneal tumour present at death. For rats 2 (K), and 5 (D), see text, pp. 240, 241.

Rat 2.—This animal was killed in the 26th week of the experiment on account of sanguineous ascites. P.M.—The liver was found to be large and pale, but no tumour was found. In places the peritoneum showed opaque patches, which under the microscope were seen to consist of a cellular layer derived apparently from proliferation of the endothelium. Subsequent observations suggest that this peritoneal thickening may have been a pre-cancerous condition.

Rat 3.—Killed in the 33rd week of the experiment on account of ascites and palpable abdominal tumours. P.M. showed: (1) a smooth, rounded abdominal tumour growing from the peritoneum in the left flank and unattached to the viscera; (2) another tumour lying between and incorporating the right kidney and liver; (3) several small nodules of growth in the peritoneum covering the intestines and accessory genital organs; (4) several thickened, opaque patches in the parietal peritoneum of the right flank; (5) a peritoneal tumour in the right loin constricting the ureter and causing hydronephrosis. Microscopical examination showed that the spindle-celled mass incorporating the liver and kidney had not invaded these organs, though it had invaded the pancreas. The peritoneal tumour in the left flank had infiltrated the subjacent abdominal muscles.

Rat 4.—Killed in the 36th week on account of gastric distension. Under anaesthesia stomach contents gushed from the animal’s mouth without any of the systemic
Mesoblastic Tumours.

Muscular movements commonly associated with vomiting. P.M.—The stomach and duodenum were greatly distended owing to involvement of the jejunum in a hard mass of growth which had incorporated the gut with the liver. There were present also tumours of the parietal peritoneum around the sites of the needle punctures, and again on the left side near the attachment of the diaphragm; opaque, thickened plaques were scattered over the peritoneum. Microscopical examination showed that some of the peritoneal tumours had begun to invade the subjacent muscles, figs. 2 and 3, Plate 11. The opaque plaques represent thickening of the peritoneum which, instead of consisting of a single layer of cells, may be a millimetre or more in depth. The liver, fig. 4, Plate 11, and the muscular coat of the jejunum had been invaded by growth.

Rat 5.—This rat died in the 37th week and was almost entirely eaten by its fellows, so that no post-mortem observations could be made.

Rat 6.—Killed in the 40th week on account of abdominal distension. P.M.—A tumour had bound the pyloric portion of the stomach, the liver and pancreas into one mass, so as to cause gastric obstruction. Microscopically the growth consisted chiefly of spindle cells. It did not appear to have penetrated the capsule of the liver or the muscular coats of the stomach, though the pancreas had been invaded. No other tumours were present. Patches of thickened peritoneum were noticeable as in the former cases.

Rat 7.—Killed in the 45th week on account of ascites and debility. P.M.—(1) A large mass of tumour surrounded the pyloric portion of the stomach, fig. 5; (2) another mass surrounded and concealed the cæcum; (3) the manubrium sterni was embedded in tumour; (4) both halves of the diaphragm were infiltrated with growth; (5) the spleen was partially embedded in and fixed to the mass which involved the stomach. In addition to these growths there were numerous nodules and plaques in the peritoneum. Microscopical sections showed invasion by growth of the stomach, fig. 6, Plate 12, the intestine and the pancreas, fig. 7.

Rat 8.—Killed in the 47th week with sanguineous ascites. Tumours were present in the stomach—both pyloric and cardiac portions—the diaphragm, liver and the adnexa of the genital organs. A peritoneal tumour had compressed the ureter and caused hydronephrosis of the right kidney. Numerous plaques and nodules of growth were scattered about the peritoneum. Microscopical examination showed invasion by tumour cells of the diaphragm, stomach, liver, and the muscles of the abdominal walls, fig. 8.

Rat 9.—Killed in the 48th week with ascites. There was a mass spreading between the viscera of the anterior part of the abdomen binding together the lobes of the liver, the stomach, pancreas, spleen and left kidney. Microscope slides showed early invasion of the pancreas. The other viscera had not been penetrated by growth. Numerous plaques were present on the parietal peritoneum, the mesentery of the bowel and the under surface of the diaphragm. The muscle of the diaphragm was infiltrated by growth, and some of the other peritoneal nodules had spread outwards amongst the superjacent muscle fibres.

Rat 10.—Killed in the 29th week on account of ascites and debility, fig. 10. P.M.—Tumours were present involving the diaphragm, liver, pancreas, stomach,
mesentery, intestine and peritoneum. The right kidney was hydronephrotic from compression of the ureter by growth. Microscopical examination showed that the diaphragm had been perforated, fig. 9, and there was invasion of the pancreas, liver, stomach, intestine and abdominal wall.

The distribution of the abdominal tumours produced in these rats is shown in a general manner in Table I.

The relative frequency with which tumours have occurred in the diaphragm is noteworthy and depends upon the distribution of the emulsion after its introduction into the abdomen. Observation has shown that an emulsion of fat, like a colloidal dye, when injected into the abdomen of a rat or a mouse, tends to pass gradually forward to the anterior portion of the cavity. In order to obtain a demonstration of this distribution a rat was given 1 c.c. of lipiodol into the right posterior quadrant of the abdomen. An X-ray photograph taken 37 days later showed that a considerable proportion of the injected material had become applied to the under surface of the diaphragm.

These peritoneal tumours are of much interest, for the development of a malignant neoplasm can be observed in every stage. The first visible response is a patchy opacity occurring here and there in the peritoneum. This opacity is due to a thickening of the membrane either by a proliferation of its own cells or by a deposit of cells upon its surface. As this thickening progresses plaques can be detected, and also nodules which give the peritoneum a warty appearance. Microscopical examination shows that many of these nodules are accompanied by a malignant infiltration of the subjacent voluntary muscle, figs. 2, 3, 8; and it has been noticed on several occasions that even in the absence of a nodule, a mere plaque is already associated with invasion of the underlying tissues. The general type of the tumours is spindle-celled, but giant cells are frequently seen, fig. 9, and occasionally there is a pronounced polymorphism. Tumours with these different cell characteristics may coexist in the same animal.

In no case has any metastasis in extraperitoneal regions been observed. Direct extension of a neoplasm into the pleural cavity through the diaphragm has occurred in two instances. The fibrous capsules and septa of the organs appear to offer the most resistance to the neoplastic invasion, but once this barrier has been passed the other tissues appear to oppose but little resistance.

Other groups of rats and mice have been treated in the same way as those of which details have been given above, but in the majority of these the experiment was started at a later date than in the completed series which forms the subject of this paper. Some abdominal tumours have occurred already in
Table I.—The Distribution of Tumours in the Abdomen.

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<td>r. kidney and liver</td>
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<td>liver</td>
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<td>Rat 6</td>
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<td>Rat 7</td>
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<td>Rat 8</td>
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<td>Rat 9</td>
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<td>Rat 10</td>
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Mesenchlastic Tumours.
these later groups and are available for further study. The results up to the present date of these additional experiments are given in Table II.

Table II.

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<tbody>
<tr>
<td>Rats</td>
<td>80</td>
<td>27</td>
<td>10</td>
<td>43</td>
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<tr>
<td>Mice</td>
<td>120</td>
<td>80</td>
<td>17</td>
<td>23</td>
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Control Experiments.

A control series of 20 rats is under observation which are receiving intraperitoneal injections weekly of the same olive oil, in the unemulsified state. At the time of writing (184th day of experiment) no tumours have been detected in the animals.

A difficulty arises in such investigations, in that the control animals bearing no tumours live very much longer than others which develop tumours, and the experiment cannot be reported as complete until the last control animal is dead, which may be a matter of years. Hence in the earlier paper upon subcutaneous spindle-celled tumours, 46 in all, in mice and rats following injection sub cutem of 1:2:5:6-dibenzanthracene in lard (Burrows, Hieger and Kennaway, 1932) we laid stress upon the fact that we were not yet prepared to define exactly the part played by the fat in the experiments described. The present state of these control experiments may be stated here.

(1) One hundred and thirty mice have been injected with the lard, and with the various mixtures of milk and lard which were being used at the same time as solvents of 1:2:5:6-dibenzanthracene. Early in the course of the experiments three of these animals in one series of 10 developed spindle-celled tumours at the site of injection; grafts from one of these failed to take. Suspicion at once arose that some confusion of the animals, or of the substances injected, in the control and other series had occurred. Since more complete precautions against any such mishap have been taken no further tumours have arisen in the controls, and no tumours have appeared except in this one series. Unfortunately, there was a high death rate among these mice, but 40 lived for more than 120 days, and 7 are alive at the time of writing (482nd day).

(2) Twenty-five rats have received frequent injections in the groin of lard
which had been sterilised at 100°; 20 of the animals are now alive (382nd day) and no tumours have been obtained.

(3) Injections in the groin have been made in mice (numbers given in brackets) of the following fats and oils; fresh pork fat (50), olive oil (10), sperm oil (10), cod liver oil (10). Some of these animals have lived for more than 400 days and no tumours have been obtained.

(4) In a further series of experiments* six spindle-celled tumours were obtained from a series of 10 rats injected sub cutem with 1:2:5:6-dibenzanthracene in lard, the last animal being killed on the 216th day; while a control series of 10 rats receiving the same lard alone had produced no tumours at the date of writing (297th day), when nine animals are still alive.

Lard heated to higher temperatures may have some neoplastic action, and further experiments upon this matter will be reported.

Discussion.

Tumours of connective tissue have now been produced at this Institute in the eight ways shown in Table III.

Table III.

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<tbody>
<tr>
<td>2. Mouse</td>
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<tr>
<td>3. Rat</td>
<td>''</td>
<td>Olive oil emulsion</td>
<td>''</td>
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<tr>
<td>4. Rat</td>
<td>''</td>
<td>''</td>
<td>Peritoneum.</td>
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<tr>
<td>5. Mouse</td>
<td>''</td>
<td>Lard</td>
<td>''</td>
</tr>
<tr>
<td>6. Mouse</td>
<td>''</td>
<td>''</td>
<td>''</td>
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<tr>
<td>7. Fowl</td>
<td>''</td>
<td>Lard</td>
<td>''</td>
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<tr>
<td>*8. Mouse</td>
<td>5:6-cyclo-penteno-1:2-benzanthracene</td>
<td>''</td>
<td>Sub cutem.</td>
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In connection with these results it is necessary to consider what part, if any, may have been played in the carcinogenesis by the fatty media as distinct from the dibenzanthracene. For the present, and pending the outcome of the control experiments mentioned above, the question whether the introduction of lard alone or of an emulsion of olive oil alone into the subcutaneous tissues or the peritoneum of a rat or mouse can determine the development of

a malignant neoplasm, must remain open. It is, of course, quite possible that
a tumour may be produced by fat alone in a susceptible animal. Cori (1927)
has recorded a spindle-celled tumour arising in the neighbourhood of a sub-
cutaneous injection of "oil" containing ovarian hormone in a mouse of a
strain liable to the development of such neoplasms. If it were found possible
to produce a large yield of tumours by the action of any fat which might occur
in the body, this result would be of greater interest than any which could be
obtained with synthetic hydrocarbons. At present we can do no more than
record the abundance of tumours which have been obtained by the use of
1:2:5:6-dibenzanthracene in fatty media.

**Summary.**

(1) Experiments are recorded in which numerous spindle-celled tumours
were induced in rats and mice by the intraperitoneal injection of 1:2:5:6-
dibenzanthracene dissolved in olive oil. The characters of the tumours are
described.

(2) The respective parts played by the hydrocarbon and fat in the carcino-
genesis have been left undetermined.

I wish to express my indebtedness to W. Davis, who has prepared the very
numerous histological specimens, and to F. Goulden and E. L. Butler for the
photographs which accompany this paper.

**EXPLANATION OF PLATES.**

**PLATE 11.**

*Fig. 2.—Rat 4.* Whole thickness of abdominal wall, from skin (below) to peritoneum
(above), showing spindle-celled nodule invading voluntary muscle. × 4.5.
*Fig. 3.—Rat 4.* Edge of peritoneal tumour shown in fig. 2. × 24.
*Fig. 4.—Rat 4.* Invasion of liver by tumour. × 84.
*Fig. 5.—Rat 7.* Tumour (T) surrounding oesophagus (O) and invading wall of stomach
(S). L = liver. × 3. See fig. 6.

**PLATE 12.**

*Fig. 6.—Rat 7.* Invasion of walls of stomach by tumour. × 21.
*Fig. 7.—Rat 7.* Early invasion of pancreas by tumour. × 21.
*Fig. 8.—Rat 8.* Invasion of voluntary muscle of abdominal wall. × 84.
*Fig. 9.—Rat 10.* Giant-celled tumour invading diaphragm. × 84.

**PLATE 13.**

*Fig. 10.—Rat 10.* Tumours of parietal peritoneum (P), diaphragm (D) [shown in fig. 9];
mesentery (M). E = residue of emulsion injected.
Further observations on Medulla oblongata of Cyprinoids. 247

REFERENCES.

Further observations on the Medulla oblongata of Cyprinoids; and a comparative study of the Medulla of Clupeoids and Cyprinoids with special reference to the Acoustic tubercles.

By H. Muir Evans.

(Communicated by Sir Henry Dale, Sec. R.S.—Received May 10, 1932.)

Introductory.

In a previous paper (Evans, 1931) an endeavour was made to correlate the feeding habits of Cyprinoids with the conformation of the medulla oblongata, and it was concluded that the Cyprinoids can be divided into three main groups.

It was found:

(1) That the vagal lobes are large in the Carps, the Goldfish and the Bream and that these are all mud feeders.

(2) That the vagal lobes and the facial lobe are small in the Roach, the Rudd, the Chub and the Dace, all of which take a fly and feed largely by sight.

(3) That the facial lobe is large in the Gudgeon, the Barbel and the Tench. The facial nerve in this group, all of which possess barbels, divides into two branches after entering the medulla oblongata. The Gudgeon and the Barbel grope and grub for their food and never take a fly.

The Tench, however, has feeding habits not unlike those of a Carp; in fact, it may be regarded as being a transitional form between the types (1) and (3).

Through the kindness of Mr. Michael Graham, naturalist on the staff of the Ministry of Agriculture and Fisheries, I have had the opportunity of