INVESTIGATION OF THE EFFECT OF 3—4 BENZPYRENE ON AMPHIBIA.

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(With 2 Figures in the text.)

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The effect of cancerogenic substances was first investigated on mammals, and it was found that they had a specific, cancerogenic effect on certain tissues and organs. Later the specific effect was determined on various species; that is, some had an inclination, others were resistant, to cancerogenic materials (Dittmar, 1939).

Amphibia were examined in respect to cancerogenic effects by Koch, G. B. Schreiber, G. Schreiber and F. Duran-Reynals (1939). Koch and Schreiber & Schreiber noted a development of neoplasm in salamanders when 3—4 benzpyrene dissolved in oil was injected subcutaneously. Neoplasm also appeared in the lungs and heart. On the other hand, in Duran-Reynal's experiments tumour was not observed when cancerogenic material was injected intramuscularly. Only necrosis of the tissues could be seen.

Because of these contradictory results we again investigated whether tumour could be produced in amphibia, or whether they were resistant.

In our experiments 3—4 benzpyrene was applied in various solutions, injected or painted on different organs. We examined not only the cancerogenic effect but also the changes in the tissues caused by histological methods. The presence of 3—4 benzpyrene in the organs was followed macroscopically under ultra violet light and in sections by fluorescence microscope.

MATERIAL AND TECHNIC.

Rana esculenta and *Triton cristatus* were used for the experiments. The following benzpyrene solutions were employed:

1.) o. 3 per cent solution of benzpyrene in acetone.

2.) o. 5 per cent solution of benzpyrene in olive oil.

3.) Colloid solution: 0.01 gr. benzpyrene dissolved, boiling, in 1 ml. glycerine, 1 drop in 1 ml. normal horse-serum, (30 drops = 1 ml.), shaken for half an hour. (GRAFFI A., 1939.)

The control and the treated animals were kept separately in glass vessels at room temperature. The height of the water was 15—20 cm. Water was changed every 3—4th day. The animals were kept from sunlight. The frogs were given no food, the salamanders were fed with meat.

The organs of each animal were examined macroscopically for pathological alterations. An ultra violet lamp was used in searching for the benzpyrene fluorescence. Heart, kidneys, liver, lungs underwent histological analysis, in some cases also the spleen, stomach, intestine, muscle and skin. The organs were fixed in 4 per cent solution of formaldehyde and were put through alcohol for paraffin embedding. The sections cut at 10 μ were stained with haematoxylineosin, Van Gieson's and Mallory's methods. One of the kidneys, a piece of the liver and of the skin were sectioned frozen, and examined, partly fluorescence microscopically, partly Sudan stained.

THE EXPERIMENTS ON THE SALAMANDER.

Treatment by painting. 7 salamanders were painted 3 times weekly with a 0.3 per cent solution of benzpyrene in acetone on the skin of the back for 4 months. About 3 to 4 drops of the solution were applied. 3 controls were painted at the same time with pure acetone.

During the experiment the skin peeled off repeatedly. The painted area had a yellowish fluorescence in u. v. light. At the end of the experiment the animal's skin showed no tumour development under histological analysis. Only necrosis could be observed, and in some places regeneration of the basal layer.

Treatment with colloid solution. 17 salamanders were injected with 0.5 ml colloidal benzpyrene solution under the abdominal skin 3 times weekly. 3 were injected with a similar solution but without benzpyrene.

In 7 salamanders, decapitated on the 30th day, pathological examination showed that the kidneys were anaemic and the liver very flabby, with a greenish-brown colour. The lungs were opalescent and anaemic. Macroscopically in u. v. light the kidneys were seen to be pitted with numerous small greenish-yellow points. Under fluorescence microscopic examination a deposit of benzpyrene was established in the cortex of the kidneys and in the endothelial cells of the renal tubules.

Under light microscope it was to be seen that the structure of the liver dissociated. The organs of the control animals showed no alteration.

The 10 salamanders examined on the 100th day had anaemic kidneys and their livers were very flabby. Under u. v. light the kidneys had a pale bluish fluorescence. The yellow points were not apparent.

Histologically necrosis and degeneration in the liver, and parenchym degeneration in the kidneys were found. The organs of the controls were normal.

Benzpyrene in olive oil solution. 7 salamanders were injected with 0.5 ml of a 0.5% solution of benzpyrene in oil, under the skin of the back. 3 were injected with the same quantity of pure olive oil.

The animals were examined on the 100th day. In the liver and kidneys the same alterations were seen as in those treated with the colloid solution. Tumour development was not observed here either.

THE EXPERIMENTS ON THE FROG.

Treatment by painting. 12 frogs were painted 3 times weekly on the abdominal skin with a 0.3% solution of benzpyrene in acetone for 3 months. 3 drops of the solution were applied. Before painting the skin was wiped dry; after the treatment the animals were kept in a dry place for half an hour or an hour. 3 controls were painted with pure acetone at the same time.

After the 3d—4th painting the skin began to peel and this went on till the end of the experiment. The skin analysed histologically showed the same effect, namely the peeling off of the epidermis on the painted area. Surrounding the painted area a normal regeneration

Fig. 1. Kidneys of a benzpyrene-treated frog in ultra violet light. Numerous small points in the kidney tissue show the characteristic yellow fluorescence of benzpyrene.

without proliferation was to be seen. The greenish yellow fluorescence of benzpyrene was noticed in the subcutis, in the glands, and in the remaining epidermis cells. The endothelium of the vein swelled and there were many erythrocytes. Peeling of the skin, and dilation of the vein was also seen in the controls. (So this is not the effect of benzpyrene but of acetone.) The yellow points on the kidneys were to be observed in u. v. light. (Fig. 1.) The urine of the animals had a yellow fluorescence too. The gall-bladder showed a blue light.

The histological picture showed a toxic haemorrhage surrounding the renal tubules. In the liver was a dissociation and a partial necrosis of the liver cells. The organs of the control animals showed no change.

Treatment with colloid solution. 23 frogs were treated with a serum-glycerine-benzpyrene solution injected 1 ml. under the abdominal skin 3 times weekly.

6 of the animals were killed on the 5th day, 10 on the 26th day and 7 between the 38th and 90th days. 5 control animals were treated similarly but without benzpyrene.

On the 5th day the decapitated frogs had a 2—3 ml. distinct opalescent exudate under the skin of the back and in the cavity of the abdomen with a greenish-yellow fluorescence. The exudate contained a few erythrocytes and some leucocytes.

In 3 cases myocardic hypertrophy was found. The structure of the lungs became loose, the fibres swollen and in the alveoles haemorrhage, peeled endothelial cells and exudative cells appeared. Haemorrhage occurred in the livers and kidneys too. Fluorescence microscopy showed the presence of benzpyrene in the cortex of the kidneys and in the endothelial cells of the renal tubules. The gall bladder had a blue fluorescence under u. v. light and the yellowish-green benzpyrene points were to be seen in the kidneys in only 3 cases.

10 frogs examined on the 26th day showed the same exudate as those of the 5th day. Hypertrophy of the heart was to be seen, and the kidneys were anaemic and showed the characteristic colour of parenchym degeneration. The livers had a greenish-brown colour and were flabby; the lungs were pale and also anaemic.

Under u. v. light the kidneys had a diffuse bluish colour with a few yellow points. This bluish colour was also found in the livers. The gall bladder had an ultramarine blue fluorescence.

In the section of the kidneys parenchym degeneration or degeneratio adiposa was noticed, as well as necrosis and toxic haemorrhage. The veins were full of erythrocytes. The structure of the livers was

dissociated, in some areas the liver cells were necrotized and in the intact areas the pigmentation of the liver was increased. The endothelium of the veins was defective. In 3 cases a special degenerative phenomenon was noticed, which was best demonstrated by MALLORY's stain method. Looking at the sections, areas of light blue colour can be seen, of 10—12 cell size, different from the bluish-red colour of the other normal areas. It is possible that in these marked light blue coloured tissue areas a chemical reaction takes place. In these marked areas are many pigment cells, and uany nuclear pyknoses and karyorhexes are to be seen.

The fibre of the lungs swelled, in the alveoli peeled endothelial cells appeared in the exudate. Benzpyrene fluorescence was to be seen only in the cortex and renal tubules.

On the 60th and 80th days 3 decapitated frogs showed changes in the lungs. The alveolar wall was voluminous and there were many cell accumulations similar to a tumour development process. But in this case it cannot be a tumour development because the cells have no malignant character. It seems to be a benign process caused by the exudate. (Fig. 2.)

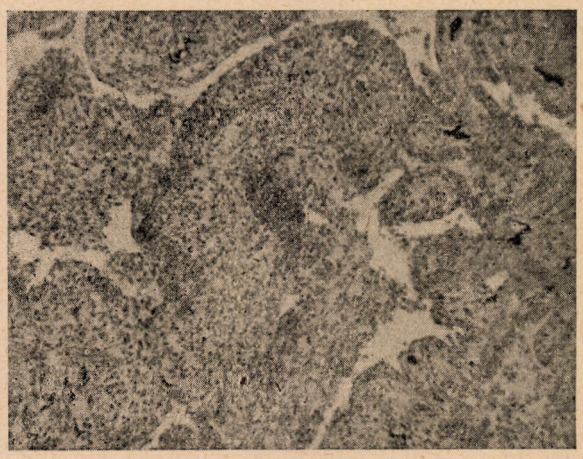

Fig. 2. Cell accumulations in the lung of a benzpyrene-treated frog.

In the other organs were changes similar to the previous series, except that here the light blue colour of the liver and kidneys under u. v. light was partial. The controls showed no alteration.

Treatment with benzpyrene dissolved in olive oil. 10—10 frogs were injected with 0.5 per cent solution of benzpyrene in olive oil. 0.5

ml was given in the adductors of the femur or under the abdominal skin. 4 controls received only pure olive oil, in the same quantity. The animals were examined on the 5th, 27th, and 70th days.

Tumour development was not observed, only a necrosis of the muscle fibre or strong hyperaemia of the skin.

Treatment with intravenous colloid solution of benzpyrene. 3 frogs were injected with 0.5 ml. serum-glycerine-benzpyrene solution intravenously. 12 hours later the strong yellowish fluorescence of the benzpyrene appeared in the kidney, the gall bladder had a weak blue fluorescence, kidneys and liver in sections showed haemorrhage.

24 hours later the fluorescence microscopic effect was the same as before,, except for the gall bladder, which had an ultramarine fluorescence.

DISSCUSSION.

To summarise, the effect of benzpyrene on amphibia is: Painting results in the epidermis cells absorbing benzpyrene but this does not cause tumour as is to be seen in the case of many mammals. Acetone causes peeling.

Subcutaneous, intramuscular, intravenous injection of colloid solution, and oil solution are absorbed in the organs and disturb the cell functions. The veins become more permeable, edema appears under the skin and in the abdominal cavity and in the lungs. The heart has to work more on account of the bad functioning of the veins and because of the haemorrhage of the organs. Thus hypertrophy of the heart occurs.

The degenerative and necrotic changes (in liver and kidneys etc.) can be taken as the known toxic effect of the cancerogenic hydrocarbons noticed in mice or other mammals *(Supniewski-Hano,* 1936, *Gaetani-Lanza,* 1937, *Maisin-Coolen,* 1936, *Parsons,* 1936, *Mauer,* 1931, *Wollbach,* 1937).

The various colours of the benzpyrene in the kidneys under u. v. light has been noticed. Benzpyrene has a greenish-yellow fluorescence in crystal form. Dissolved in colloidal solution it retains its colour, but in alcohol solution the blue colour appears. Higher temperature also changes the colour. (Weigert F., Mottram J. C., 1940). In our investigations it was to be seen that the kidney tissues adsorb benzpyrene and at first the benzpyrene in them has a yellow fluorescence. Later, when the tissue degenerates, it changes to blue. Thus physico-chemical

changes occur in the benzpyrene molecules during the degeneration of the kidney tissue.

It is to be mentioned too that in no case was fluorescence noticed in the depot fat of the frogs, though the great affinity of benzpyrene for fatty tissue is well known.

On the basis of our experiments we agree with F. DURAN-REYNALS, that benzpyrene does not cause tumours in salamanders and in frogs, but causes a very strong toxic effect in the cells and organs.

SUMMARY.

3—4 benzpyrene does not induce tumours in *Triton cristatus* and *Rana esculenta,* but it produces a toxic effect in their bodies. It produces a toxic haemorrhage in the kidneys and liver, parenchym degeneration, fatty-degeneration and necrosis. In frogs it produces edema in the lungs, exudative cells and an edemic swelling of the alveolar wall. Edema forms under the skin and in the abdomen. Benzpyrene is excreted by the liver and kidneys.

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