decomposes to give 4-hydroxydiphenylamine and diphenylamine under the conditions used in the acidic and enzymatic hydrolysis. Although diphenylamine was not detected in the urine hydrolysates, it is uncertain whether the original urine contains conjugates of the 4-hydroxy- or the N-hydroxy-compound or both.

A male rabbit was dosed orally with an aqueous suspension of 5 g of diphenylamine in divided doses of 1 g over a period of nine days. 4-Hydroxydiphenylamine and trace amounts of 2-hydroxydiphenylamine and of diphenylamine could be detected in ether extracts of the unhydrolysed urine. Hydrolysis of the ether extracted urine with β-glucuronidase also gave these compounds.

The potassium salt of the sulphate ester of 4-hydroxydiphenylamine was isolated from rabbit urine in the following manner. After treatment of the urine with lead subacetate and removal of the excess lead with hydrogen sulphide, the filtrate was adjusted to pH 7 and concentrated to small volume under reduced pressure. On cooling the concentrated extract deposited a crystalline compound which was recrystallized from alcohol containing a small amount of aqueous potassium hydroxide. This substance was identified as follows. (a) Preliminary tests showed the presence of nitrogen, sulphur, and potassium and hydrolysis with hydrochloric acid gave 4-hydroxydiphenylamine. (b) The infra-red and ultra-violet spectra of this compound (Found: C, 45.77; H, 3.32; N, 4.10. $C_{12}H_{10}NO_4SK \cdot {}^1/_2H_2O$ requires C, 46.13; H, 3.22; N, 4.48) showed a general similarity to 4-hydroxydiphenylamine. (c) Careful examination of the infra-red spectrum in acetonitrile showed a weak band at 3350 cm^{-1}, characteristic of –NH– adsorption and almost identical to a band obtained with authentic diphenylamine (3355 cm^{-1}). The nuclear magnetic resonance spectrum in dimethylformamide showed the exchange of active hydrogen and the presence of nine aromatic protons when the sample was treated with deuterium oxide, which further substantiates the existence of a free –NH– grouping in the molecule and shows that the phenolic substitution occurs on the ring.

The structure of this compound was confirmed by synthesis according to the method of FEIGENBAUM and NEUBERG[7]. The infra-red spectrum of the synthetic material (Found: C, 44.61; H, 3.97; N, 3.93. $C_{12}H_{10}NO_4SK \cdot H_2O$ requires C, 44.84; H, 4.05; N, 4.36) was identical with that of the metabolite. Since the melting point of this sulphate ester was not characteristic, the S-benzyl-iso-thiuronium salt of both the synthetic compound (m.p. 119–121°C) and the metabolite (m.p. 118–120°C) was prepared. No depression in melting point occurred on admixture of the two salts and the infra-red spectra were identical.

The isolation of the potassium salt of the sulphate ester of 4-hydroxydiphenylamine indicates direct hydroxylation of the aromatic ring followed by conjugation. If the sulphate ester of N-hydroxydiphenylamine were formed, followed by rearrangement either *in vivo* or during the isolation procedure, the *ortho* isomer would be expected by analogy with the rearrangement of phenylhydroxylamine-*o*-sulphonic acid under acidic conditions to *o*-aminophenol and *o*-aminophenyl hydrogen sulphate *in vitro*[8]. Phenylhydroxylamine-N-sulphonic acid has been shown to rearrange to *p*-aminophenol and *p*-aminophenyl hydrogen sulphate under acid conditions *in vitro*[8], and this sulphate ester could potentially arise from such a rearrangement. However, direct conjugation of the aromatic amino group with sulphuric acid is not a common reaction *in vivo*. Experiments are in progress to quantitatively determine the metabolism of diphenylamine in the rat and rabbit and to determine whether N-hydroxylation occurs by isolation and characterization of the glucuronide fraction.

Zusammenfassung. Nach oraler Verabreichung von Diphenylamin wurde das Kaliumsalz des 4-Oxydiphenylaminsulfatesters aus dem Harn des Kaninchens isoliert. Die enzymatische Hydrolyse des Kaninchenharns ergab kleine Mengen von 2-Oxydiphenylamin, die mit Hilfe von Dünnschicht-Chromatographie identifiziert wurden. Bei der Ratte gelang nach intraperitonealer Verabreichung von Diphenylamin bei enzymatischer und saurer Hydrolyse des Harns der chromatographische Nachweis des 4-Oxydiphenylamins als Hauptprodukt der Umwandlung.

W. E. ALEXANDER,
A. J. RYAN, and S. E. WRIGHT

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Effect of Feeding Benzpyrene on Reproduction in the Rat[1]

SAVKUR et al.[2,3] have studied the effect of methylcholanthrene on the developing mouse embryo. They injected methylcholanthrene directly into the embryonic fluid of 10-day-old embryos and observed abnormalities at birth and resorption of other embryos. In studies in which benzpyrene crystals either were mixed with food or given by capsule to dogs, mice, chickens, ducks and cockroaches[4–7], a blue fluorescence of the skin and viscera occurred. Benzpyrene was demonstrated spectrophotometrically in the tissues. The blue fluorescence rapidly regressed when the benzpyrene was discontinued. In the first experiment with chickens, the eggs developed a bluish fluorescence and were infertile. However, in later studies the eggs were fertile[6]. In view of the apparent effect of benzpyrene on the fertility of eggs, an experiment was devised to study the effect of benzpyrene on preg-

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nancy in the rat. Our observations are reported at this time.

Methods and Material. The rats were virgin females 90 days of age obtained from the Holtzman Rat Company. Six males of proven fertility and of the same strain were used. The control animals were fed Purina laboratory chow. The experimental group was fed laboratory chow to which was added 1 mg of benzpyrene per gram of food. The technique for preparing this ration has been described[7]. Food and water were given *ad libitum*. The rats were kept either in individual cages or in small groups. A male was placed with a female on the evening of proestrus and removed on the following morning. If sperms were found in the estrus smear on the morning after mating, it was considered as day 1 of pregnancy.

The rats were weighed at intervals during the experiment. Vaginal smears were made for 11 days before the rats were placed on the benzpyrene-containing food in order to eliminate any animals with abnormal cycles. Regular cycles were observed in all the rats.

During the experiment the rats were observed for fluorescence with an UV-lamp (Aloe No. 52140, UV-mineralight, high intensity, long wave, 3660 Å). The viscera were observed similarly when the rats were killed. A complete autopsy was made on each animal. Multiple sections were removed for histologic study. These tissues were fixed routinely in a 4% solution of formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin.

Results. Experiment 1: There were 8 females fed the benzpyrene-containing ration and 6 the laboratory chow. The average weight of the control group was 220 g and the test group 227 g. The females in the control group were mated with benzpyrene-fed males and the benzpyrene-fed females were mated with the control males.

Daily vaginal smears were recorded for each animal for a 28-day period beginning on the first day of the feeding of benzpyrene. During this 28-day period all rats of both groups, with one exception, continued to exhibit regular estrous cycles. The 6 control animals had a total of 36 days of estrus and the 8 animals of the test group 48 days of estrus. The only irregularity in the cycles occurred in one of the rats of the test group that showed a 12-day diestrous period. This was interpreted as a period of pseudopregnancy and it was probably induced by the trauma of the vaginal smear procedure.

Following these observations, three successful matings were obtained within a two-week period among the 6 control females and 5 successful matings among the 8 females fed benzpyrene. In addition to the presence of sperm in the vaginal smear, successful pregnancies in these animals were signaled by the onset of continuous diestrous vaginal smears. The 3 pregnant animals in the control group showed the usual outward signs of advancing pregnancy and all delivered their litters without difficulty on the 22nd day of pregnancy. These 3 females had litters of 3, 6 and 11 young and all of the young appeared normal grossly. When first observed, all pups were alive except for one which was either stillborn or died soon after delivery.

Of the 5 females which became pregnant in the benzpyrene-fed group only one delivered. This animal delivered 4 pups on the 23rd day of pregnancy and only 2 of these were born alive. One of the stillborn pups was grossly malformed (Figure). The other 3 appeared normal grossly, although they were small. One of the 2 surviving pups was killed and the other one was left with the mother. This latter pup died, obviously of starvation, three days after birth. The mother showed no signs of lactating. Three of the remaining 4 pregnant females in this group delivered no young, although vaginal bleeding was observed in each rat on the 23rd or 24th days of pregnancy. During the latter half of pregnancy it was clear from outward appearances that these animals would not deliver normal litters. A hysterectomy was performed on the fifth animal on the 20th day of pregnancy and 6 fetuses which appeared grossly normal were removed.

Experiment 2: After the litters were weaned the three control females were mated a second time. In this experiment the 6 control females were mated with control males. Three successful matings were obtained in the group of control females. Matings were made between benzpyrene-fed males and 7 benzpyrene-fed females; however, only 2 pregnancies resulted. During a two-week period of time prior to these matings, all of the females (with the exception of one benzpyrene-fed animal which now lacked uterus and ovaries) continued to exhibit regular estrous cycles.

After the termination of this second experiment, 2 of the 3 pregnant control animals delivered 8 and 11 normal-appearing pups. The third animal was killed on the 20th day of pregnancy and had 10 normal fetuses. Both of the benzpyrene-fed females that became pregnant were killed on the 22nd day of their pregnancies. The experiment was terminated at this time and an autopsy was carried out on all of the animals, except for two pregnant females in the control group.

At autopsy it was found that fetal resorption had occurred in one of the pregnant females in the benzpyrene-fed group; the other female had 4 dead fetuses in the right uterine horn. This was in contrast to the pregnant animal in the control group that showed 10 living and normal-appearing fetuses in the uterine horns.

UV-Fluorescence. Normally the skin of the rat has a faint blue fluorescence, while that of rats fed benzpyrene fluoresces a deep blue. The viscera of normal rats did not fluoresce, but the viscera of rats fed benzpyrene were blue. The liver, lungs, and testicles of the group fed benzpyrene fluoresced a light blue color; however, there was no fluorescence of the heart or spleen. The first portion of the stomach in the treated group of rats fluoresced a deep blue, while the stomach of the controls fluoresced red.

One rat fed benzpyrene was killed on the 23rd day of pregnancy. There were 4 dead pups in the right and none in the left horn of the uterus. The amniotic fluid was blood-tinged. The pups were blue-black in color and were

The mother was fed benzpyrene during the period of gestation. Four pups were delivered on the 23rd day of pregnancy. One pup was malformed, one either was a stillborn or died shortly after birth, and two were normal. These pups were smaller than pups from a female fed the control ration.

edematous. When observed with UV-light the uterine wall, the placenta, the fetal membranes and the pups fluoresced blue, while the placenta from a normal rat had a red fluorescence.

Pathologic Study. There were no tumors observed in this group of rats. The 6 non-pregnant females that had been fed the chow containing benzpyrene for $3\frac{1}{2}$ months averaged 253 g, while the 3 non-pregnant controls averaged 270 g. No significant differences were observed histologically in the ovaries, adrenals or pituitary gland of the treated and the control groups of rats. Several of the rats had an acute and chronic pneumonitis. No pathologic changes were observed in the viscera of the benzpyrene-treated rats that could be associated with this hydrocarbon. No attempt was made in this study to observe fluorescence in the cells of the benzpyrene-treated rats, and no significant histologic lesions were observed.

Discussion. It may be concluded from this experiment that the feeding of benzpyrene to rats does not interfere with the ovarian cycles, ovulation, fertilization, or implantation; however, it does have a deleterious effect on embryonic development. It may interfere with lactation, although more data is needed to establish this point.

Only one pup was observed to be malformed. This abnormality may or may not be related to the presence of this hydrocarbon. In several of the pregnancies the fetuses apparently died and were absorbed. The fetuses removed at autopsy were dead in one female fed benzpyrene. These observations would support the opinion that either benzpyrene or a metabolite may be lethal for developing embryos.

The blue fluorescence as observed in the viscera of the rats fed benzpyrene is similar to that previously described in ducks, chickens, dogs, mice, and cockroaches fed benzpyrene[4–7]. It is not known at this time whether this blue fluorescence results from the presence of benzpyrene or a metabolite. In previous studies of mice fed large amounts of benzpyrene the liver and kidneys were blue and benzpyrene was demonstrated spectrophotometrically.

The fetuses from mothers fed benzpyrene had a blue fluorescence. The uterine wall, fetal membranes and the placenta likewise fluoresced blue, while similar tissues in rats fed the control rations had a red fluorescence. The agent responsible for this blue fluorescence is transmitted through the milk to the young mouse[4].

Obviously the amount of benzpyrene fed to this group of rats is excessive. We have not observed, however, any evidence of toxicity, other than in the developing embryo. WHITE and WHITE[8] in 1939 expressed the opinion that certain of the carcinogenic hydrocarbons were toxic and suggested that the growth-inhibitory substances exert their effects by the production of a specific deficiency in the sulfur-containing amino acids, probably through the requirements of the organism for organic sulfur in the form of cystine and methionine, for detoxication mechanisms.

RIGDON and GIANNUKOS[9] observed a loss of weight in mice fed methylcholanthrene, pyrene, anthracene and benzpyrene. This loss of weight was shown to be related to the amount of food consumed. Mice ate little of the food containing large amounts of these hydrocarbons. No pathologic changes were observed in the viscera of the mice fed benzpyrene and methylcholanthrene. The rats fed benzpyrene in the present experiment had no pathologic changes that we could associate with a toxic effect resulting from the benzpyrene. HADDOW and ROBINSON[10] and HADDOW et al.[11] did not observe histologic changes in the liver, spleen, kidney, bone marrow, adrenals, or thyroid of mice given benzpyrene intraperitoneally.

PAYNE[12] injected rats intraperitoneally with benzpyrene and observed cysts in the ovary, endometrial hyperplasia of the uterus, and the development of mammary adenocarcinomata. The interstitial (Leydig) cells were completely absent except in those animals where interstitial cell adenomata occurred. CRAMER and HORNING[13] found no changes in the endocrine organs of mice painted with benzpyrene. LARIONOW[14] observed changes in the endocrine organs of mice painted with benzpyrene that were similar to those occurring in old age. PEACOCK[15] did not observe any benzpyrene in the developing mouse embryo when pregnant mice were injected intravenously with benzpyrene.

Résumé. Lorsque des rats sont soumis à une diète riche en benzopyrène, leurs embryons peuvent dégénerer et être résorbés. Le placenta et les membranes fétales ont une fluorescence bleue. Normalement, ces mêmes tissus ont une fluorescence rouge.

R. H. RIGDON and E. G. RENNELS

Departments of Pathology and Anatomy, The University of Texas Medical Branch, Galveston (U.S.A.),
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Slow Periodicity in the Dark Discharge of Retinal Units

During experiments concerned with the statistical parameters of retinal discharge under conditions of complete dark adaptation, an almost regular periodic firing pattern was sometimes observed in retinal ganglion cells.

Cerveau isolé cats (postcollicular transection) were used. Eye movements were prevented by a curare-like drug (Sincurarine, Farmitalia) and the animal was maintained under artificial respiration. The cornea was excised and the lens ablated. A steel microelectrode 5–10 μ in diameter was introduced into the retina by a micromanipulator. The potentials picked up in this way were amplified and recorded on a multichannel magnetic tape, Ampex F.M. 1107 with band pass filters set from 0 to 5000 cps. In some of the preparations EEG activity and arterial blood pressure were controlled.