

carden-(20:22)-olid) als Steroidkomponente im Conjugat 4 vorkommt, spricht gleichfalls für Glukuronsäure als Conjuganden, weil die Glukuronyl-Transferase offenbar nur mit α -ständigen OH-Gruppen an C-3 von A/B *cis*-Steroiden reagiert (erschlossen aus dem ausschliesslichen Vorkommen der entsprechenden Metabolite von Steroidhormonen¹³).

Die Beobachtungen insgesamt erlauben den Schluss, dass der Metabolit 4 mit 3-Epi-digitoxigenin-glukuronid identisch ist. Vermutlich werden im tierischen Organismus auch andere Cardenolidgenine, die durch Epimerisierung entgiftet werden können¹⁴, über anschliessende Konjugation mit Glukuronsäure ausscheidungsfähig gemacht.

Summary. After incubation of digitoxigenin with slices from livers of man, rabbit or dog, a total of 5 chloroform-insoluble, highly polar metabolites has been isolated by

extraction with ether-ethanol. Two of the products were the sulfuric acid esters of digitoxigenin or 3-epi-digitoxigenin, as demonstrated earlier, and two further ones could not be identified. The fifth metabolite appeared to be identical with 3-epi-digitoxigenin glucuronide.

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¹³ R. REUBER und J. SCHMIDT-THOMÉ, in HOPPE-SEYLER/THIERFELDERS *Handbuch der pathologisch- und physiologisch-chemischen Analyse*, 10. Auflage (Springer-Verlag, Berlin-Göttingen-Heidelberg 1955), Bd. III/2, p. 1451.

¹⁴ K. REPKE, Proc. First Intern. Pharm. Meeting, Stockholm 1961 (Pergamon Press, Oxford 1963), vol. III, p. 47.

Absorption and Excretion of Benzpyrene in the Cockroach (*Periplaneta americana*)¹

We have reported² that the skin and viscera of chickens and mice have a blue fluorescence following the oral administration of benzpyrene. Benzpyrene is present in the blood of chickens and ducks and in the bile of chickens and dogs thus treated. According to WILLIAMS³, this blue fluorescence may result either from benzpyrene or one of its metabolites. CHAMBERS and KIRBY⁴ and KOTIN et al.⁵ found that benzpyrene and its metabolites are normally eliminated through the liver and kidney. In our present study of absorption and elimination of benzpyrene in the cockroach, the benzpyrene was added to the food. Fluorescence was observed with ultraviolet light and benzpyrene was extracted from the tissues.

Methods and Material. Adult cockroaches (*Periplaneta americana*) were caught locally and put into large glass jars. Laboratory chow was used as food, the pellets being crushed and put on the bottom of the jars. Water was available in small containers. Crystals of benzpyrene were added to the crushed pellets of laboratory chow in concentrations of 1, 5, 10, and 20 mg of benzpyrene per g of food. This food was again molded into pellets weighing 1 to 2 g and dried in an oven for 24 h at 78°C.

The length of time the roaches were fed the benzpyrene-containing food varied from 24 h to 2 months. In some experiments the roaches were fed benzpyrene-containing food for 24 h and then normal laboratory chow for varying periods. Some of the roaches were kept in individual jars in order to obtain specimens of feces to test for benzpyrene. For this test a single pellet of the feces was put into a small test tube to which 1 to 3 ml of benzene was added. The tube was shaken briefly and then observed for blue fluorescence with an ultraviolet light (Aloe No. 52140, ultraviolet mineralight, high intensity, long wave 3660Å). A blue fluorescence was interpreted as suggestive of the presence of benzpyrene. Absorption curves for benzpyrene were made on a Beckman spectrophotometer on some of these stool specimens. Some of the pellets of feces were observed for benzpyrene crystals under a dissecting microscope with ultraviolet light.

The roaches were anesthetized with ether, put into small jars, and covered with benzene. The bodies were then macerated to determine whether or not benzpyrene was present. Sometimes only the legs of roaches were

removed for extraction. After remaining in the benzene for intervals of 1 to 24 h, the supernatant fluid was removed and observed with ultraviolet light. An absorption curve for benzpyrene was obtained on many of these specimens, using the Beckman spectrophotometer.

Two additional hydrocarbons, pyrene and anthracene, were added to the food in a concentration of 5 mg/g of laboratory chow. This was fed to 2 groups of roaches. On the 6th day the food containing these hydrocarbons was discontinued and the roaches were fed the standard ration. Some of the roaches were killed. Pyrene and anthracene were extracted from the tissues in benzene using the same technique as previously described for extracting benzpyrene from the body of the roach.

In some of the experiments the cockroaches were anesthetized, put into the freezing compartments of a refrigerator, and kept for varying intervals before they were either photographed or put into benzene for extraction. Roaches were observed and photographed using the ultraviolet light. Eastman's Ektachrome daylight type film with a 2A gelatin filter was used for all colored transparencies. Black and white photographs also were made using ultraviolet light.

Results. Roaches readily eat laboratory chow containing benzpyrene. There has been no evidence that benzpyrene is injurious to the roach when fed for a period of 2 months. Stools were positive for benzpyrene within 8 h following the feeding of benzpyrene-containing food. Control specimens always were negative for benzpyrene. Numerous yellow crystals, consistent with benzpyrene, were present in stools of treated roaches. The stools remained positive for benzpyrene for approximately 72 h when they were obtained from insects fed benzpyrene-containing food and then changed to normal laboratory chow (Figure 1).

Benzpyrene is extracted readily from the body of roaches fed benzpyrene-containing food (Figure 2). Benz-

¹ Supported by grant CA 01469-11 from the National Cancer Institute, Public Health Service.

² R. H. RIGDON and J. NEAL, Texas Rept. Biol. Med. June (1963).

³ R. T. WILLIAMS, *Detoxication Mechanism*, 2nd ed. (John Wiley and Sons, Inc., New York 1959), p. 222, 224.

⁴ J. G. CHAMBERS and A. H. M. KIRBY, *Biochem. J.* **34**, 1191 (1940).

⁵ P. KOTIN, H. L. FALK, and R. BUSSE, *J. Natl. Cancer Inst.* **23**, 541 (1959).

pyrene persists in the body of the roaches for varying periods of time after the benzpyrene-containing food has been replaced with normal food. A moderate amount of benzpyrene was obtained on the 16th day from roaches fed 10 mg of benzpyrene-containing food for 24 h and then standard food for 16 days, but no benzpyrene was observed in roaches similarly treated and killed on the 27th day. There was a decrease in the amount of benzpyrene obtainable from the tissues corresponding to the interval elapsing between the time that the benzpyrene-containing food was discontinued and the time of the extraction of benzpyrene. The quantity of benzpyrene obtainable from the tissues also was influenced by the quantity of benzpyrene in the food and the amount of food consumed. Hungry roaches eat more than those routinely fed. Such factors naturally would influence the amount and duration of demonstrable benzpyrene in the tissues. Benzpyrene was demonstrable in the tissue extracts of roaches following the time that the stools were negative (Figure 1).

The ventral surface of roaches fed benzpyrene for 24 h fluoresced with a blue color (Figure 3). This blue fluorescence gradually increased. The blue fluorescence was most conspicuous in the tissues about the mouth, eyes, ventral surface of the neck and thorax, in the small pads (setae) on the legs and in the tissues about the anus. When the wings and the posterior portion of the skeleton were removed, the soft tissues within the body cavity showed a deep blue fluorescence.

Pyrene and anthracene were present in the bodies of roaches killed on the 6th experimental day after having been fed the pyrene and anthracene-containing foods. These hydrocarbons also were demonstrated in the bodies of roaches killed on the 7th day following the discontinuation of the hydrocarbon-containing food (Figures 4 and 5), but none was found in roaches killed on the 17th day.

Discussion. It is obvious from these experiments that the cockroach absorbs benzpyrene from the gastrointestinal tract and it is readily extracted from the body and legs. Benzpyrene is present in stools of roaches fed benzpyrene-containing food. Benzpyrene can be extracted from the roaches several days after their stools are free of

this hydrocarbon. Only small amounts of benzpyrene, however, are demonstrable in the bodies of these roaches 15 days after food containing benzpyrene has been discontinued and none is found in roaches examined on the

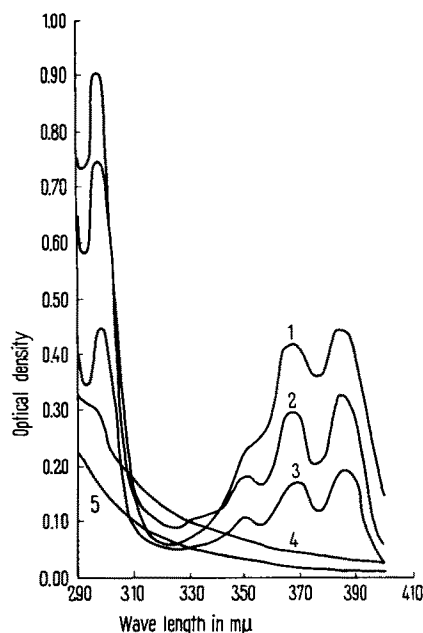


Fig. 2. Absorption curve for benzpyrene in tissues of roaches. Curves 1, 3, 4 benzpyrene, 5 micromes/ml in benzene. Tissue extracted in benzene Beckman DU Spectrophotometer 2. Body of roaches fed benzpyrene for 8 days (10 mg/g food) 3. Legs of roaches fed benzpyrene for 24 h 10 mg/g food) 4. Body of roaches fed normal food 5. Legs of roaches fed normal food

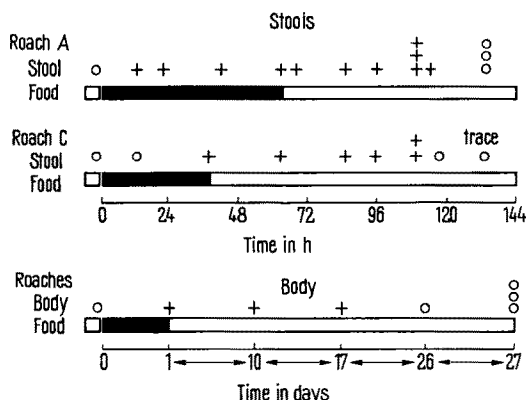


Fig. 1 shows interval in which stools from roaches are positive when fed benzpyrene and the time in which benzpyrene is present in the tissues after the food containing benzpyrene was discontinued.

- Interval when benzopyrene containing food was fed (5 or 10 mg benzopyrene/g of laboratory chow)
- Interval when laboratory chow was fed
- + Benzopyrene present
- o Benzopyrene absent

Body of roach extracted in benzene and absorption curve for benzpyrene read on Beckman DU Spectrophotometer.

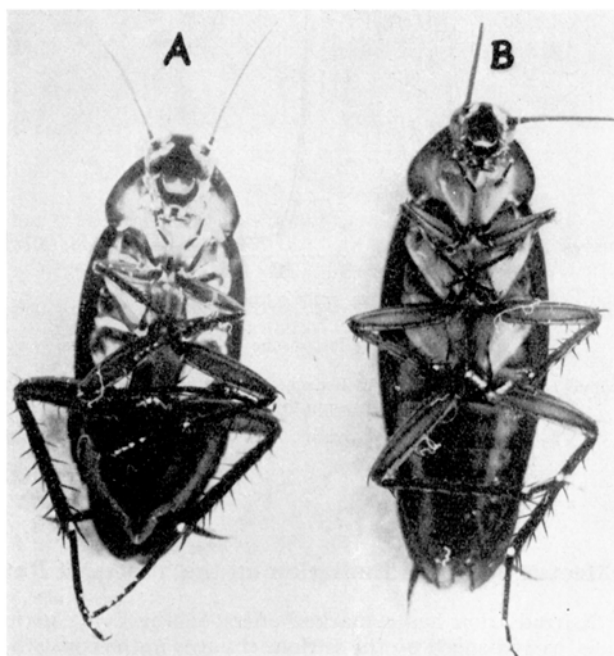


Fig. 3. A. The ventral surface of a roach fed 10 mg of benzpyrene per g of laboratory chow for 20 days. The area has a blue fluorescence with ultraviolet light. In this photograph the area is white. B. Control roach photographed with ultraviolet light.

27th day. These observations would indicate that the roach eliminates benzpyrene following oral administration as we reported in the mouse, chicken and duck². However, the rate of excretion is much slower in the roach than it is in the warm blooded animals. Certainly, there is nothing in these experiments to suggest that benzpyrene is stored in the tissues of the roach for long periods of time.

Although there is some blue fluorescence of the wings and body of the untreated roach, benzpyrene was never extracted from the body. Furthermore, the intensity of the blue fluorescence is minimal in the controls as compared with that of the roaches fed benzpyrene.

WILLIAMS³ observed that benzpyrene, as well as some of its metabolites, has a blue fluorescence. No attempt, however, has been made in this study to demonstrate any metabolites that may be present in either the tissues or stools. Obviously, benzpyrene is metabolized within the roach since no benzpyrene was demonstrated in the stools after the food containing benzpyrene had been discontinued. However, it was still present in the body of roaches, although subsequently it disappeared. Benzpyrene remains in the tissues of the roach much longer than it does in the tissues of the mouse and chicken. These observations in the cockroach would suggest that tissues other than the kidney and liver may metabolize benz-

pyrene since there is no liver or kidney in the cockroach like in mammals.

Pyrene and anthracene can be extracted from the tissues of roaches fed these hydrocarbons. Pyrene and anthracene, like benzpyrene, will persist in the tissue of the roach after the food containing these 2 hydrocarbons has been discontinued. Neither of these hydrocarbons was demonstrated in the tissues of the roaches killed 17 days after food containing pyrene and anthracene had been discontinued⁶.

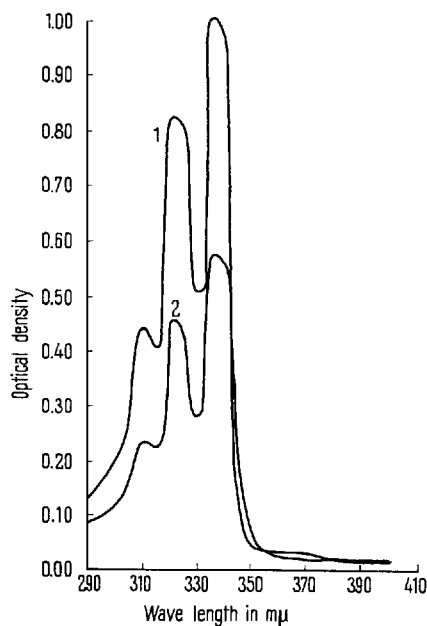


Fig. 4. Absorption curve for pyrene in tissues of roaches.

Curve 1. Pyrene standard in benzene. Tissue extracted in benzene Beckman DU Spectrophotometer

2. Body of roach fed pyrene 6 days (5 mg/g food) then control food for 7 days.

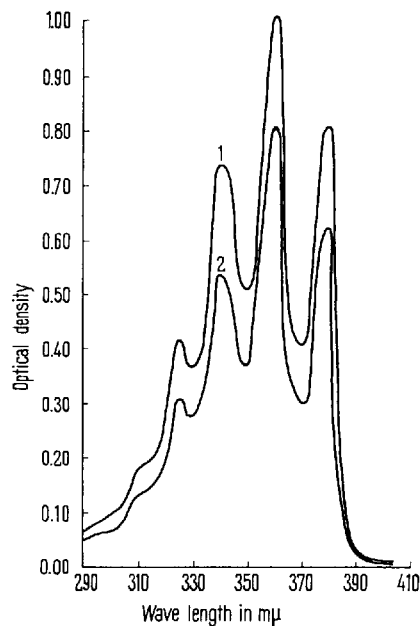


Fig. 5. Absorption curve for anthracene in tissues of roaches.

Curve 1. Anthracene standard Read in benzene. Tissue benzene extracted Beckman Spectrophotometer

2. Body roach fed anthracene 6 days (5 mg/g food) then control food for 7 days.

Résumé. Le benzopyrène le pyrène et l'anthracène peuvent être révélés au moyen du spectrophotomètre dans les extraits de tissu des blattes (*Periplaneta americana*) nourries avec ces hydrocarbures; ils sont excrétés lorsqu'on interrompt l'alimentation.

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⁶ This study in the cockroach was suggested by J. MACK, a laboratory assistant, who also contributed much to the technical conduct of the experiment.

Effect of Ionizing Radiation on the Testes of Rat

X-irradiation has a marked effect on the living germ cells, as evidenced by the various changes in the constitution and behaviour of the intracellular inclusions. The sequence of these aberrations is difficult to follow as chronological studies of the living irradiated germ cells have not been carried out in the present investigations and will be dealt with in detail elsewhere.

Pure bred Wistar rats, eight weeks old, were subjected to whole body irradiation according to the following schedule—325 r at 200 kV, filter 1 Al plus $\frac{1}{2}$ Cu, target distance 50 cm, with the dose rate at 36 r/min. The initial dose of 325 r was repeated every 24 h till the maximum dose reached 1300 r. Control rats from the same age group were kept and provided with the same experimental conditions without exposure to X-rays. Induced rats from different dose levels were sacrificed after a week and