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Absorption, Distribution, Metabolism, and Excretion of Insect-Metamorphosing Hormone Ecdysterone in Mice. II[1]

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The absorption, distribution, and excretion of ^{3}H-labelled ecdysterone were studied in mice by means of autoradiographic technique. When administered intraperitoneally, ecdysterone was rapidly and easily absorbed into the blood and distributed to various organs (in particular, in the excretory organs such as the liver, gall bladder, intestine, and kidney) in which the radioactivity reached the maximal levels quite soon. Its clearance from the organs excluding the excretory organs was rather rapid, and the radioactivity specifically accumulated in the liver was gradually transported into the intestinal lumen *via* the gall bladder which showed the highest and continuous uptake. The glandular secretion of radioactivity into the gastric mucosa also partially contributes to its transportation from the blood to the digestive tract. After oral administration of ^{3}H-ecdysterone, the absorption from the intestine was slow and limited and high radioactivity in the gastro-intestinal tract was retained. The liver took up some radioactivity which attained a maximum soon after administration and gradually decreased due to the biliary excretion through the gall bladder in which the radioactivity hit the maximum level about 1 hr after administration and maintained a high level thereafter. Elimination of the administered ecdysterone from the body appears to be fast and its excretion pattern indicates that the excretion in the feces, mostly derived from the biliary excretion, is much more important than that in the urine after both intraperitoneal and oral administration.

Ecdysterone is a hormone which regulates the moulting during the growth and development of arthropods. Recently it has been found that ecdysterone shows a number of interesting physiological activities on higher animals as well as arthropods.[3] For the occurrence of such pharmacological and biochemical effects of ecdysterone on higher animals, it must be first absorbed into the body, distributed to target organs, and taken up into cells at the site of action. It is therefore of interest and of importance to study the behavior of ecdysterone in the body.

In the preceding paper, we[1] have investigated the absorption, distribution, and excretion of ^{3}H-ecdysterone by scintillation counting of mouse organs after intraperitoneal and oral administration. As a result, it was found that ecdysterone is much more easily absorbed from the peritoneum than from the gastro-intestinal tract, that after absorption it is selectively distributed to the excretory organs (*inter alia*, to the liver), and that it is excreted mainly in the feces rather than in the urine. However, the number of organs studied to determine uptake of radioactivity was limited, and details of the distribution within an organ or tissue could not be investigated. The present work was performed in order to clarify the distribution pattern and excretion pattern of ecdysterone more conclusively by means of systematic autoradiographic studies.

Experimental

Labelled Compound——Preparation of [23,23,24,24-^{3}H]ecdysterone was described previously.[1] The specific activity used for the autoradiography was 1.43 mCi/mg and the radiochemical purity was corroborated by giving a single radioactive spot on a thin-layer chromatogram.

1) This paper is Part XVI in the series on Steroids. Part XV: H. Hikino, Y. Ohizumi, and T. Takemoto, *Yakugaku Zasshi*, **92**, 945 (1972).
2) Location: *Aoba-yama, Sendai.*
3) *cf*, H. Hikino and T. Takemoto, *Naturwissenschaften*, **59**, 91 (1972).

Autoradiography——Male mice of dd strain weighing about 20 g were used. For injection, the radioactive ecdysterone was dissolved in a concentration corresponding to 5 mCi/ml physiological saline and 0.2 ml (1 mCi/0.7 mg) of the solution was injected intraperitoneally into a mouse. For oral administration, the radioactive ecdysterone was dissolved in a concentration to 5 mCi/ml water and 0.2 ml (1 mCi/0.7 mg) of the solution was ingested orally through a metal stomach tube into the mouse after 12 hr starvation. Ten min, 1,4,8,24, and 72 hr after administration, the mice were anesthetized with ether and immersed in a mixture of solid carbon dioxide and acetone at about $-70°$.

The autoradiographic technique employed was based on that described by Matsuoka.[4] Thus, after a

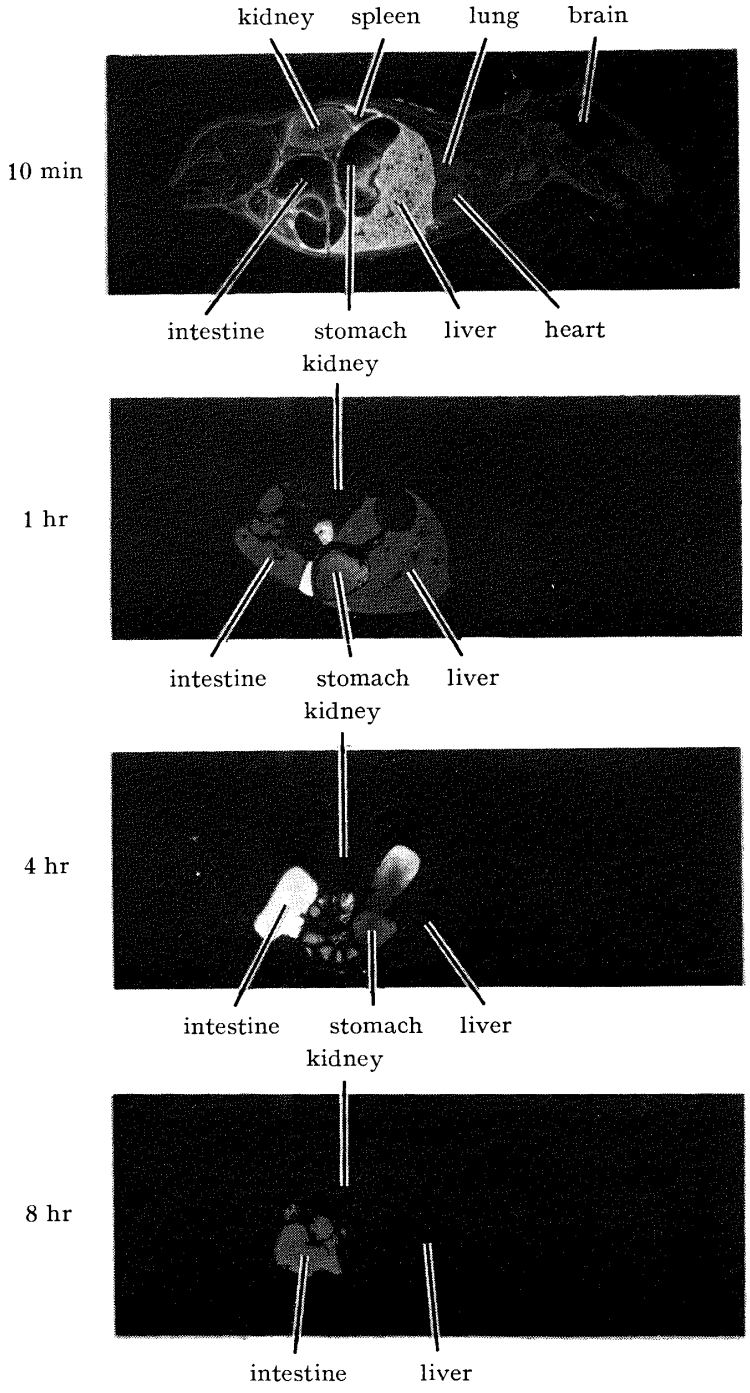

Fig. 1. Autoradiograms showing the Distribution of Radioactivity in Mice at various Intervals after intraperitoneal Injection of ^{3}H-Ecdysterone

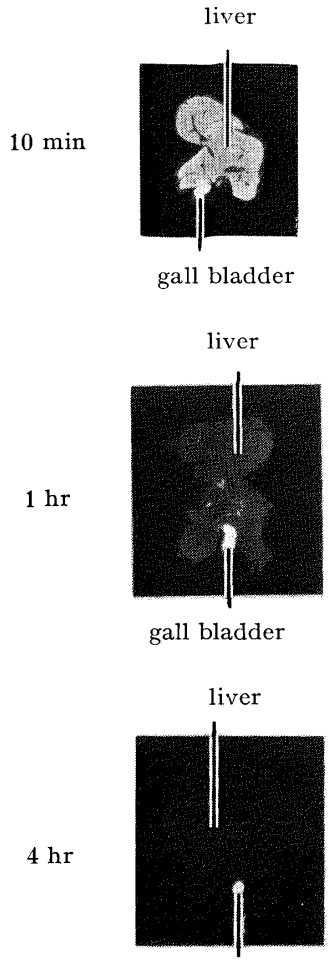

Fig. 2. Autoradiograms showing the Distribution of Radioactivity in Mouse Livers at various Intervals after intraperitoneal Injection of ^{3}H-Ecdysterone

4) O. Matsuoka and M. Kashima, *Radioisotopes*, **14**, 195 (1966).

frozen mouse was embedded on a microtome stage with aqueous carboxymethyl cellulose gel, sagittal 40 μ sections through the whole animal were cut by means of tape-sectioning with a heavy microtome in a freezing room. For the examination of the distribution in the gall bladder, the mice were sacrificed at suitable intervals after administration, and the livers were extracted, coated and embedded on a microtome stage with aqueous carboxymethyl cellulose, and dipped in a solid carbon dioxide–acetone mixture, sagittal 40 μ sections being cut through the gall bladder in the manner as described above. After being dried at $-15°$ for 24 hr, the sections were brought into contact with Sakura Type N industrial X-ray film and exposed for 2 months.

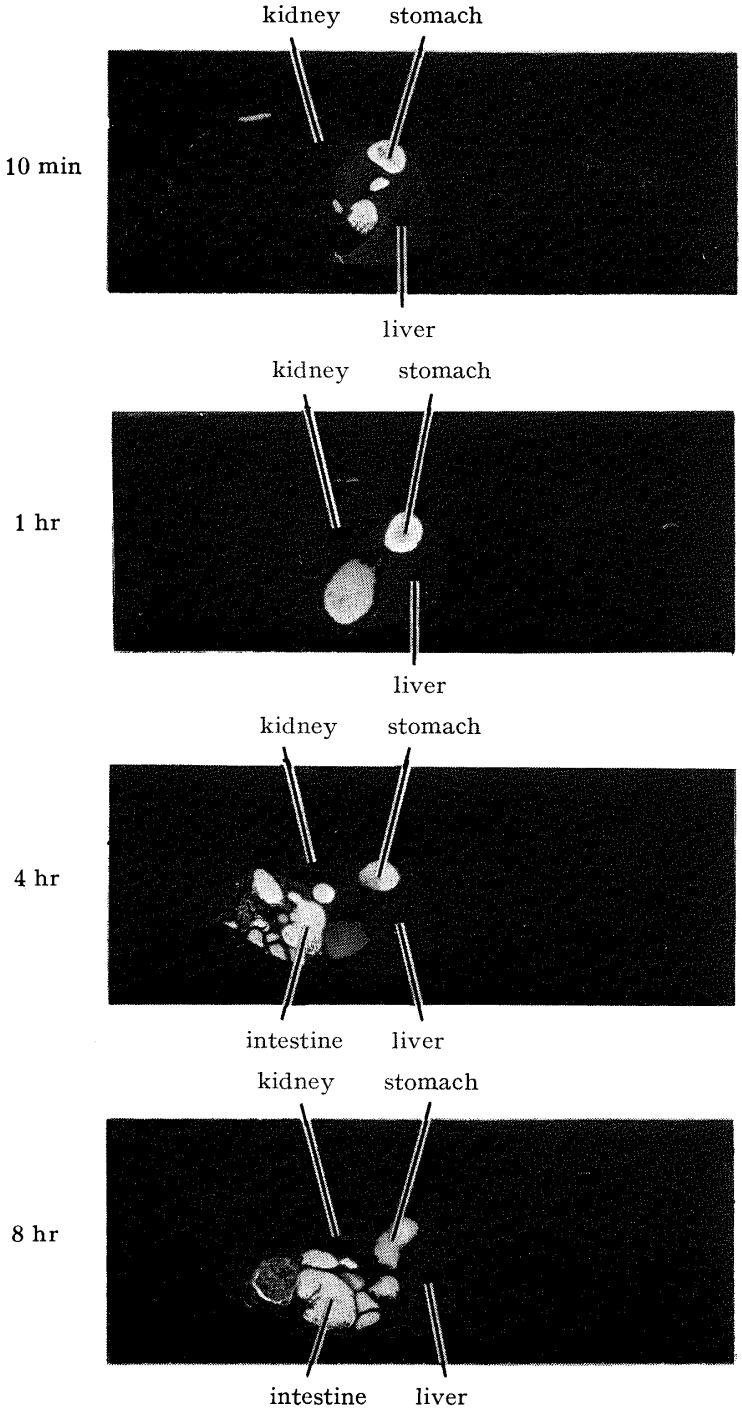

Fig. 3. Autoradiograms showing the Distribution of Radioactivity in Mice at various Intervals after oral Administration of ^{3}H-Ecdysterone

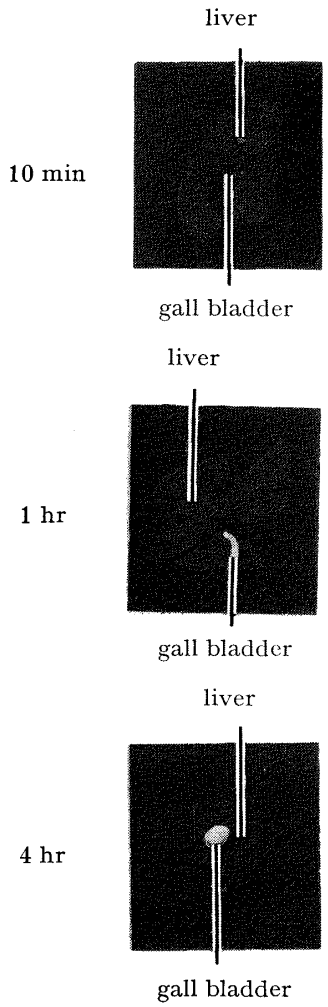

Fig. 4. Autoradiograms showing the Distribution of Radioactivity in Mouse Livers at various Intervals after oral Administration of ^{3}H-Ecdysterone

Result

Representative autoradiograms from mice at various time intervals after intraperitoneal administration are shown in Fig. 1 (whole body) and Fig. 2 (liver).[5] At 15 min after administration, a wide distribution of radioactivity was observed throughout the body tissues. In particular, the highest radioactivity was shown in the gall bladder, and the liver also showed a strong radioactivity. The contents of the stomach and the cortex of the kidney were considerably labelled, and a little uptake of radioactivity was seen in the lung and skin. The concentration in the skeletal and cardiac muscles was very low, and no appreciable radioactivity was detected in the brain. At 1 hr after administration, the radioactivity in most tissues disappeared and the remaining radioactivity was concentrated in the liver, gall bladder, gastro-intestinal tract, and kidney. Up to 4 hr after administration, the gall bladder kept a high level of radioactivity and the concentration in the liver decreased with time, while that in the gastro-intestinal contents increased. At 8 hr after administration, some radioactivity was observed in the intestinal contents, and the liver and kidney were weakly labelled. At 24 hr after administration, most of the radioactivity disappeared from the body.

Whilst autoradiograms from mice at various time intervals after oral administration are exemplified in Fig. 3 (whole body) and Fig. 4 (liver). At 10 min after administration,the greatest radioactivity was concentrated in the stomach and some radioactivity was observed in the liver and gall bladder. During the period from 1 to 8 hr after administration, the radioactivity in the digestive tract had gradually migrated from the stomach to the intestine, while that in the liver showed a gradual decrease and that in the gall bladder maintained a high level. At 8 hr after administration high radioactivity still remained in the gastro-intestinal contents. Most of the radioactivity in the body disappeared before 24 hr.

Discussion

The present study on the distribution of ecdysterone in mice revealed that by intraperitoneal injection of ^{3}H-ecdysterone a wide distribution of radioactivity was observed throughout the body tissues and the tissue concentrations of radioactivity reached their maximal levels quite soon after administration, demonstrating that ecdysterone is easily absorbed from the peritoneal route and is rapidly taken up by the tissues through the blood. A high accumulation of ^{3}H-ecdysterone in the liver immediately after the intraperitoneal injection has already been observed by scintillation counting in mouse organs and it was suggested that an active transport mechanism is involved.[1] The autoradiographic pictures presently obtained immediately after injection were characterized also by the rapid appearance of a high radioactivity in the liver, coinciding with the previous results. Further, specific accumulation of the highest radioactivity soon after injection was noted in the gall bladder, indicating that a remarkably rapid excretion of the radioactivity from the liver to the intestine through bile had already begun. A concentration which exceeded the blood level immediately after injection was found also in the kidney from which radioactive materials were later excreted in the urine. The concentrations of radioactivity in various organs thereafter declined rather rapidly, with no organ or tissue retaining appreciable radioactivity for more than 1 hr except for the excretory organs such as the liver, gall bladder, gastro-intestinal tract, and kidney. Thus, the gall bladder showed the highest concentration of radioactivity throughout the observation period. In support of this observation, the concentration of radioactivity in the liver gradually declined, while that in the intestinal contents increased with the passage of time, reaching a maximum about 4 hr after injection, and decreasing thereafter due to the fecal excretion. These observations indicate that the most part of the absorbed ecdysterone

5) White areas in the autoradiograms were radioactive.

is cleared from the circulation by the liver and carried into the intestinal tract *via* bile. Doubt may now be raised as to whether the radioactivity accumulated in the intestinal tract was derived only from the biliary excretion or whether the glandular secretion of radioactivity from the blood circulation was participating simultaneously. The finding that from 10 min to 4 hr after injection a fairly high radioactivity was seen in the gastric contents shows that a direct grandular secrtion of ecdysterone into the gastric mucosa and contents from the circulating blood also plays a part.

After oral administration of ^{3}H-ecdysterone, it was found that the radioactivity was mainly restricted to the gastro-intestinal tract. Thus, for the period from 15 min to 8 hr, most of the radioactivity was still located in the gastric and intestinal contents, indicating that the absorption of ecdysterone from the digestive tract is slow and limited. In addition to the gastro-intestinal tract, a high radioactivity was distributed in the excretory organs such as the liver and gall bladder in which the extent of accumulation in the former was much lower than in the case of intraperitoneal injection, and nevertheless that in the latter continued to be high. These observations demonstrate that the rate of absorption from the intestine is limited and, furthermore, as has been observed in the case of intraperitoneal administration, ecdysterone and its metabolites accumulated in the liver are rapidly but continuously brought back into the intestinal lumen through the biliary excretion.

It was noted that a significant amount of radioactivity was still present in the intestine at 8 hr after oral administration while only a small amount of radioactivity remained there at the same period after intraperitoneal administration. This difference is considered to arise from the relatively slow transport of ecdysterone from the stomach to the duodenum upon oral administration, compared to the rapid transport from the peritoneum to the duodenum *via* the liver and gall bladder upon intraperitoneal administration.

Concerning the excretion pattern of ecdysterone, the present autoradiographic study together with the previous finding that after either intraperitoneal or oral administration on mice a larger amount of radioactivity was recovered in the feces (91% or 64%, respectively) than in the urine (5% or 11%, respectively),[1)] indicate that the biliary excretion contributes significantly much more than the urinary excretion.

In conclusion, it can be stated that on both *i.p.* and *p.o.* administrations of ^{3}H-ecdysterone the radioactivity is taken up by the liver in a high concentration, the distribution mostly restricted to the excretory organs such as the liver, gall bladder, gastro-intestinal tract, and kidney, and the excretion is performed mainly in the feces through the biliary excretion.

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