Whole-body Autoradiographic Study of Vitamin K Distribution in Rat

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Vitamin K group is known as an anti-haemorrhagic vitamin and has a common skeleton of 2-methyl-1,4-naphthoquinone. There are several homologs which differ in the alkyl side chain at 3-position of naphthoquinone ring. Of these homologs, vitamin K\textsubscript{2(20)} (K\textsubscript{2}) which has a geranyl-linalyl side chain is considered to be a physiologically active type of this vitamin, since Martius, et al.\textsuperscript{2}) reported that the administered vitamins K is converted to this type of homolog in pigeon chicken and rat.

We reported the tissue distribution and metabolic fate of K\textsubscript{2} by isotopic tracer techniques.\textsuperscript{3}) However, the determination of radioactivity by combustion method\textsuperscript{4}) is technically difficult for several tissues. In the present paper, we report the distribution of K\textsubscript{2} in rats by whole-body autoradiography which is advantage in determining the intuitively fine distribution of radioactivity. And the results are compared with those of naturally occurring vitamin K\textsubscript{1} and synthetic vitamin, vitamin K\textsubscript{3}.

We also studied the bile excretion of these vitamins, because of their less urinary excretion and high radioactivity in intestinal content.

Material and Method

Materials—Vitamin K\textsubscript{1}-(2-methyl-\textsuperscript{14}C) (3.3 mCi/mmmole) and vitamin K\textsubscript{2(20)}-(2-methyl-\textsuperscript{14}C) (4.3 mCi/mmole) were synthesized from menadione-(2-methyl-\textsuperscript{14}C) with specific radioactivity of 5.8 mCi/mmole which was purchased from Radiochemical Center (England).

Vitamin K\textsubscript{2(20)}-(6-\textsuperscript{3}H) (41.2 mCi/mmole) was synthesized from 2-methylnaphthalene-(6-\textsuperscript{3}H) which was obtained by tritiation of 2-methyl-6-bromonaphthalene.\textsuperscript{5})

Animals—Male rats of Wistar strain, weighing from 100 to 110 g were used for the whole-body autoradiography and these of 170 to 190 g for bile excretion experiment.

Preparation of Whole-body Autoradiogram—The autoradiogram preparation was carried out by the method of Matsuoka, et al.\textsuperscript{6}) Each \textsuperscript{14}C labeled vitamin K was dissolved in water with nonionic detergent, HCO-60, and the volume corresponding to 0.1 μCi/g of body weight of each sample was injected into the tail vein. At a definite period of time, the rat was killed with chloroform and fixed with acetone—dry ice. Thin sections were prepared with a microtome 1111 model (Yamato Koki Co., Ltd.) and contacted with Sakura N type X-ray film for 2 weeks. The exposed film was developed with Konidol (Konishiroku) for 4 min and fixed with Konifix (Konishiroku Co., Ltd.) for 20 min at 20°C.

Measurement of Biliary Excretion—The biliary cannulation was performed in rats under ether anesthesia and 1 μmole of each sample (\textsuperscript{14}C labeled K\textsubscript{1}, K\textsubscript{2} and \textsuperscript{3}H labeled K\textsubscript{2}) was injected intraperitoneally. The bile juice was collected every 2 hours.

\textsuperscript{1}) Location: 3-20-1 Kitashinjuku Shinjuku-ku Tokyo.
\textsuperscript{4}) S. Baba, S. Iwahara, and H. Ogino, Yakugaku Zasshi, 88, 635 (1968).
\textsuperscript{5}) T. Konishi, S. Baba, and T. Matsuura, Radiisotope, 20, 31 (1971).
\textsuperscript{6}) O. Matsuoka and M. Kashima, Radioisotope, 15, 196 (1966).
Result and Discussion

Previous report revealed that the K₂ administered orally or intraperitoneally was concentrated in lungs, liver, pancreas and adrenal gland shortly after the administration and then spread over the whole body. These results, however, were obtained by determining the radioactivity in the isolated tissues by combustion method. The radioactivity in several tissues, such as lymph node, bone marrow, and intestinal mucosa could not be determined by this method because of the technical difficulties. The autoradiography technique gave us informations of more fine distribution in these tissues.

Fig. 1 shows the whole-body autoradiogram of K₁ at 1 hr and 24 hr after the administration. The highest radioactivity of K₁ is found in the liver 1 hr after administration.

Adrenal gland, lungs, bone marrow, kidney, and lymph node subsequently show rather high radioactivity. Spleen also shows the radioactivity. After 24 hr, the radioactivity tends to spread over the whole body. While the radioactivity in blood and liver decreases, it is recognized that the radioactivity in adrenal gland, spleen, gastrointestinal mucosa and pancreas is higher at 24 hr after the administration than at 1 hr.

On the other hand, the 1 hr autoradiogram of K₂ given in Fig. 2 shows the highest radioactivity in intestinal content. Liver also shows as higher radioactivity as in the case of K₁.
Fig. 2. Whole-body Autoradiogram of Vitamin K$_{2(20)}$-(2-methyl-$^{14}$C) 1 hr (upper two) and 24 hr (lower two) after Intravenous Administration at 1 hr. The radioactivity in lungs, spleen, adrenal gland, kidney, and bone marrow is high next to that in liver. Even 1 hr after the administration, a higher K$_{2}$ incorporation than K$_{1}$ is found in gastrointestinal mucosa and spleen compared with the heart blood of each radiogram as standard. The distribution pattern of K$_{2}$ at 24 hr is very similar to that of K$_{1}$. There seems to be no essential difference in the distribution pattern at 24 hr after the administration between K$_{1}$ and K$_{2}$. The decreasing rate of K$_{2}$ in liver seems to be much faster than that of K$_{1}$ but the residual radioactivity in intestinal content after 24 hr is high. These results are consistent with the data in which the turnover rate of K$_{2}$ in liver is faster than that of K$_{1}$.

The autoradiogram of K$_{3}$ is shown in Fig. 3. Because the urinary excretion of K$_{3}$ which is about a half of the administered dose during the first 24 hr, is faster than K$_{1}$ and K$_{2}$, the autoradiogram at 24 hr could not be obtained under this experimental condition. It appears that the tendency of K$_{3}$ to spread over the whole body is faster than the other two homologs, but the total incorporation is not so high. The highest radioactivity of K$_{3}$ is found in the

The biliary excretion of these vitamins K was also studied, because the autoradiogram of these homologs showed rather high radioactivity in intestinal content. Fig. 4 shows the biliary excretion of vitamins K after intraperitoneal injection. During the first 12 hr, only 8.5% of administered dose was excreted in the case of K1, while K2 and K3 were more recovered than K1, being 74.1% and 36.6% respectively. These results are consistent with those of autoradiogram which showed a rather high radioactivity in intestinal content 1 hr after the administration of K2 and K3, while relatively low radioactivity is found in the case of K1. It is interesting that both homologs, K2, which differs from K1 only in the number of double bond in the side chain at 3-position of naphthoquinone ring, and K3, which has no side chain, are excreted in bile much more than K1.

The present experiments revealed some difference between the behavior of K1 and that of K2 in rat as follows: the clearance of K2 from liver is faster than that of K1, radioactivity in intestinal content of K2 is higher than that of K1 after 24 hr, the incorporation of K2 in gastrointestinal mucosa and spleen is higher than that of K1, but there seems to be no essential difference in the distribution pattern between K1 and K2 24 hr after the administration.

There seems to be a positive relationship between the liver clearance and the biliary excretion rate in K1 and K2, and so it would be interesting to compare the biliary metabolites of K1 and K2. Otherwise, it is also in good agreement with other experimental results7,8)

that vitamin K₃ is excreted in urine faster and that the tissue incorporation is low compared with the other two homologs.

It is also interesting that vitamin K is incorporated into the tissues such as spleen, lymph node, and bone marrow which are responsible for lymphatic cell generation, but the relatively high radioactivity found in lymph node 1 hr after the administration may be related to the lymphatic absorption of these vitamins.⁹)

The autoradiogram presented a good advantage for finding the radioactivity in intestinal mucosa, lymph node, and brown fat in which determination of radioactivity by combustion method had some technical difficulties.


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A New Diosgenin Glycoside, Aspidistrin, from *Aspidistra elatior* Blume

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The air-dried underground parts of *Aspidistra elatior* Blume (Liliaceae) (Japanese name, haran) have been known in Japan as a folk medicine,²) for instance as expectorant, diuretic and tonic. Concerning their steroidal constituents Takeda and his coworkers reported³) diosgenin, a sapogenin (markogenin –), a phytosterol and an unidentified amorphous saponin in the material collected in February.

This paper describes the isolation of a new diosgenin glycoside named aspidistrin (I) and the characterisation as 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (3-O-[β-lycotetraoside).

An examination of the underground parts collected in April for the steroidal constituents showed the existence of β-sitosterol and stigmasterol in a free state or as esters and of diosgenin along with a trace amount of unknown compound in the acid hydrolysate of the glycoside fraction. Therefore the major glycosidal steroidal compounds in the plants were thought to be the glycosides of diosgenin or the corresponding furostanol derivatives.⁴)

Extraction and separation of the glycosides in conventional way as shown in Chart 1 gave a fraction (Fr. 4) which afforded on recrystallization a pure compound (I) as colorless needles, mp 265–267° (decomp.), [α]D 68°. I was accompanied in the Fraction A by several more polar compounds which were positive to the Ehrlich reagent and presumed to be furostanol bisglycosides.⁴) The Fraction C consisting of the Ehrlich positive compounds was

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