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MOUSE PLACENTAL TRANSFER OF F^{18} IN COMPARISON WITH Ca^{45}

by

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In a previous paper (*Ericsson & Malmnäs* 1962) a review of the literature as well as quantitative experiments with F^{18} in humans and rabbits demonstrated the limited permeability of the placenta to fluoride ions. Survey autoradiograms using the same isotope in pregnant mice had shown earlier that there was at least a considerable retardation of the fluoride transfer to the foetuses of these animals (*Ericsson & Ullberg* 1958, 1959, *Appelgren, Ericsson & Ullberg* 1961). These investigations also showed that the fluoride in the placenta was sometimes concentrated to small spots which coincided with argyrophilic areas in the sections, when stained according to von Kossa. Those areas were supposed to be the well-known degenerative calcifications formed during the last part of pregnancy.

The aim of the present investigation is to visualize more thoroughly the mouse placental transfer of fluoride ions, as F^{18}, in comparison with the calcium transfer, as Ca^{45}.

AUTORADIOGRAPHIC STUDY

Material and methods

Isotopes

F^{18} was produced by neutron irradiation of $LiNO_3$, enriched to about 95 % Li^6, in the uranium reactor R 1 of Atomenergi Company, Stockholm. The 2-step reaction produces H^3, F^{18} and some contaminating activity, mainly Na^{24}. For removal of H^3 and contaminating activity the irradiated salt was dissolved in water and calcium phosphate was precipitated in the solution. F^{18} was taken up almost quantitatively in the precipitate, which also included some H^3. The precipitate was centrifuge-washed with distilled water, transferred to a microdistillation apparatus and distilled with $HClO_4$ at about 140°, using a slow air stream. A distillate of about 4 ml was taken up in 0.5 ml 0.15-N NaOH and concentrated by evaporation to about 0.5 ml. After neutralization to pH 5—8 the concentrate consisted of a carrier-free solution of F^{18} of physiologically compatible ionic strength and low remaining activity of H^3, and with no other detectable contamination. This solution was used for intravenous injection.

$Ca^{45}Cl_2$ (spec. act. > 1 C/g Ca) was obtained from O.R.N.L., Oak Ridge, Tenn., U.S.A. and was diluted with saline to the desired concentration for i.v. injection.

The widely diverse decay rates (half life of F^{18} = 110 min., and of Ca^{45} = 153 days) were utilized for the selective autoradiographic registration of the two isotopes (*Appelgren, Ericsson & Ullberg* 1961).

Whole animal F^{18} autoradiography

Full term pregnant white mice (1—2 days before expected parturition) weighing 30—40 g were used as experimental animals.

Most of the animals were injected in a tail vein. The dose given was 0.25—0.5 ml of the F^{18} solution and contained 1—5 μC/g body weight. These animals were sacrificed at 3, 4, 15, or 60 minutes post-injection.

One animal was given a peroral dose of F^{18} and Ca^{45} simultaneously 30 min. before being sacrificed. This mouse had been fasting for two hours before the ingestion by stomach syringe of 0.8

ml solution containing about 140 μC F^{18} and 2 μC Ca^{45}, both practically carrier-free.

After deep ether anaesthesia the animals were rapidly embedded in a 0°C solution of carboxy methyl cellulose in water on a microtome stage, which was immersed into a mixture of hexane and dry ice (— 75°C). The whole bodies were thus rapidly frozen directly on the stage and ready for sectioning.

The frozen animals were sectioned in a cold-room (— 10°C). 20 μ sagittal sections, including placental tissues and foetal and maternal hard tissues, were taken according to the technique of *Ullberg* (1954).

Apposition autoradiograms were made by pressing the sections against Structurix (Gevaert) X-ray film. In order to avoid chemical fogging of the emulsion, the autoradiographic exposure, which lasted over night, was carried out in the cold (— 10°C).

The sections of the animal that was given both F^{18} and Ca^{45} were first exposed to one emulsion for 3 hours for the registration of F^{18}. After an interval of about 24 hours, during which time F^{18} decay is practically complete, the same sections were apposed for 3 weeks on a second emulsion to obtain the Ca^{45} autoradiograms. A 5 hour control exposure of some sections was made on the second day in order to ascertain that the low Ca^{45} dose did not visibly interfere with the registration of F^{18}.

After exposure the sections were removed from the emulsion which was developed with ordinary technique. The sections were freeze-dried at — 10°C before being stained and mounted in Euparat (Platters & Garnett Ltd, Manchester, England). The autoradiographic technique was largely the same as has earlier been described by *Ullberg* (1958).

Detailed F^{18} + Ca^{45} autoradiography of placenta and foetuses

Four full term pregnant white mice (1—2 days before expected parturition) were used as experimental animals. The radioisotopes were administered in a tail vein.

The F^{18} dose was 10—15 μC/g body weight and the Ca^{45} dose 0.05 μC/g body weight.

Thirty minutes after the injection the whole uterine parcel was removed under ether anaesthesia and mounted with carboxy

methyl cellulose on a microtome stage. Then all of it was frozen by immersion for some minutes into a mixture of hexane and CO_2-ice (— 75°C).

5 μ serial sections were taken of the placentae and the foetal tissues in a refrigerated room (— 10°C). The sectioning was made according to the technique of *Ullberg* (loc. cit.), Scotch tape No. 688 being used as a backing for the sections.

After drying for one hour at — 10°C every second section was mounted on Ilford G 5 nuclear emulsion plate (5 μ emulsion thickness).

An acetone solution of urea alkyd, to which was added a hardener (8 + 1 + 0.3 parts), was used as an adhesive for this mounting. The hardener was composed of equal parts of ethylene glycol and absolute alcohol, to which was added 1 % conc. hydrochloric acid. After addition of the hardener, the urea alkyd solution can be kept for about 24 hours at room temperature.

The emulsion plates were dipped in this adhesive and placed vertically in order to let surplus solution run off. After some minutes, a dry sticky surface was obtained to which the section adhered well. The urea alkyd layer was found to have a uniform thickness of 0.6 μ when measured in the interference microscope.

After three hours' exposure, the tape backing consisting of polyvinyl chloride was removed from its adhesive by pressing a sponge soaked in acetone against the tape for 5 minutes. The tape adhesive was then removed by immersion in xylene for 1½ hours, after which the xylene was replaced by alcohol and the preparation taken through an alcohol series (100 %, 96 %, and 70 %). With the sections attached the plates were then developed for 5 minutes (Gevaert 230) and fixed (Gevaert 305 A).

The sections were finally stained either with haematoxylin-eosin, or according to the van Gieson method, and the sections and plates mounted in Canada balsam.

After about 24 hours, when the F^{18} content had disintegrated, the remaining sections were applied to G 5 emulsions according to exactly the same technique but with 3 weeks' exposure time in order to produce the Ca^{45} pictures. Developing, staining and mounting of emulsion plates and sections, respectively, were performed in the same way.

Some of the sections were similarly applied to G 5 plates and exposed for 5 hours on the second day in order to ascertain that the low Ca^{45} dose was not able to cause any contamination of the F^{18} picture.

Results

At three and four minutes after the injection the maternal hard tissues already showed a strong uptake of F^{18}. The concentration of F^{18} in the maternal blood was still high and the placentae showed about the same concentration as the blood. No uptake was visible in the foetuses (Fig. 1). Fifteen minutes after the injection most of the radioactivity was concentrated in the maternal hard tissues (Fig. 2). The maternal blood had a very low con-

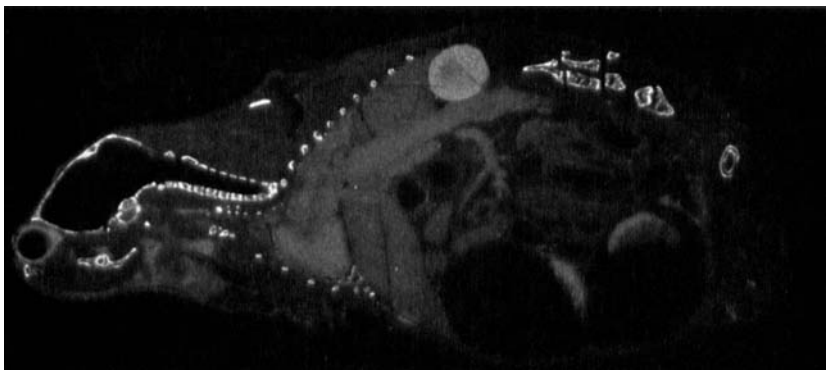


Fig. 1. F^{18} distribution in pregnant mouse 4 minutes after intravenous injection.

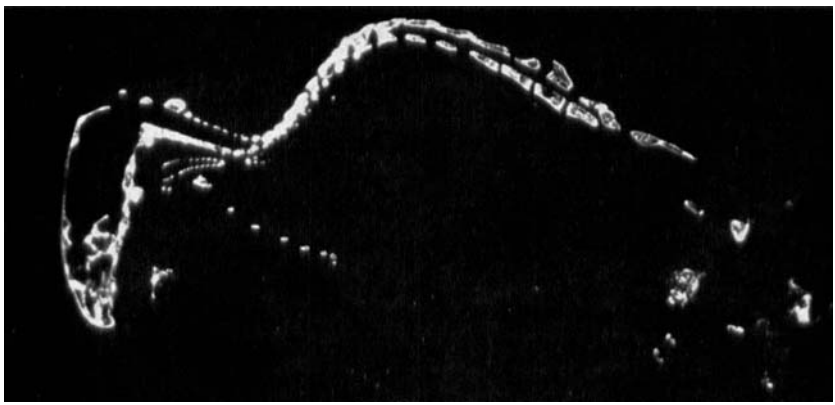


Fig. 2. F^{18} distribution in pregnant mouse 15 min. after intravenous injection. F^{18} uptake in placental spots and foetal mineralized tissues barely discernible.

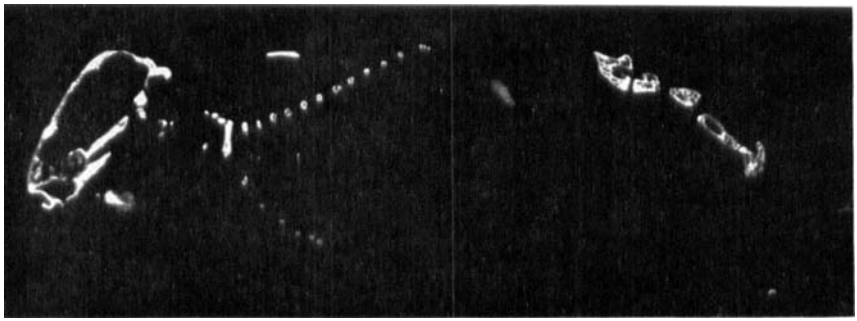


Fig. 3. F^{18} distribution in pregnant mouse 30 min. after intravenous injection. Foetal skeletons still barely discernible on original autoradiograms.

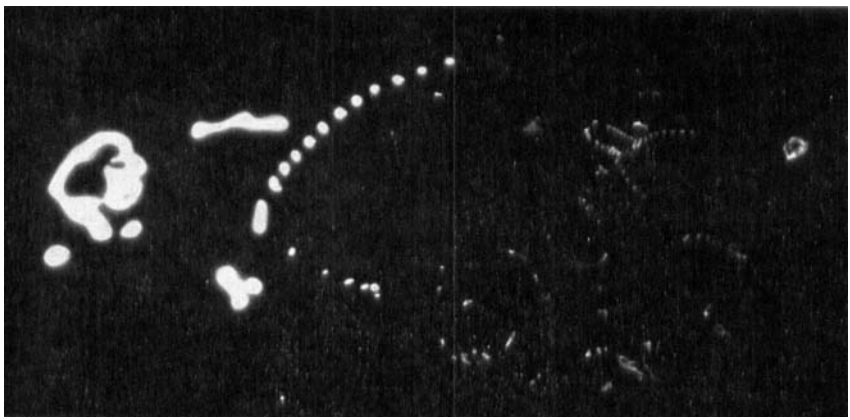


Fig. 4. F^{18} distribution in pregnant mouse 60 min. after intravenous injection. Mineralized parts of foetal bones clearly visible but with much lower activities than maternal bones (overexposed on this picture).

centration and so had the placentae, except for some small spots of high activity. In the foetuses, a very faint picture could be seen indicating an uptake of F^{18} in the hard tissues.

Thirty and sixty minutes after the injection the maternal soft tissues and the placentae showed a still lower radioactivity with the exception of the spots mentioned above (Figs. 3, 4). The foetal hard tissues showed a stronger uptake compared to the picture after fifteen minutes, but still very weak compared to the maternal skeleton.

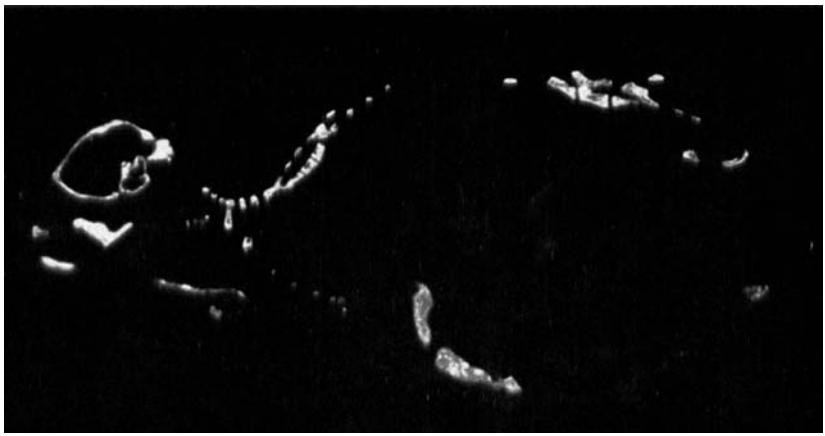


Fig. 5. F^{18} distribution in pregnant mouse 30 min. after peroral ingestion of F^{18} + Ca^{45}. Foetal skeletons hardly discernible.

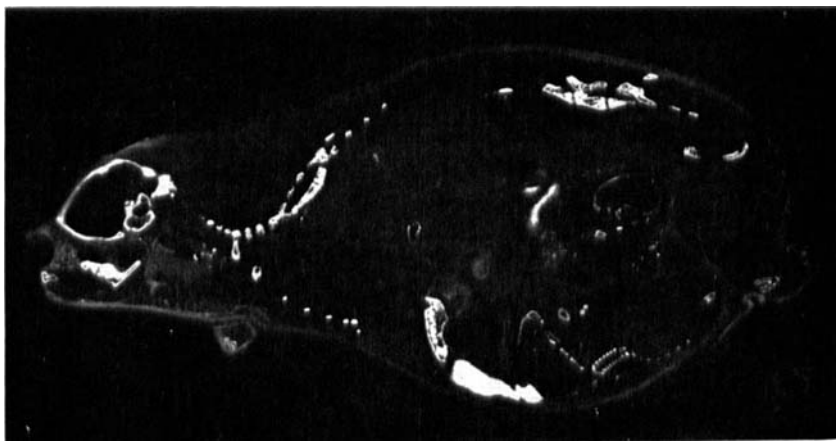
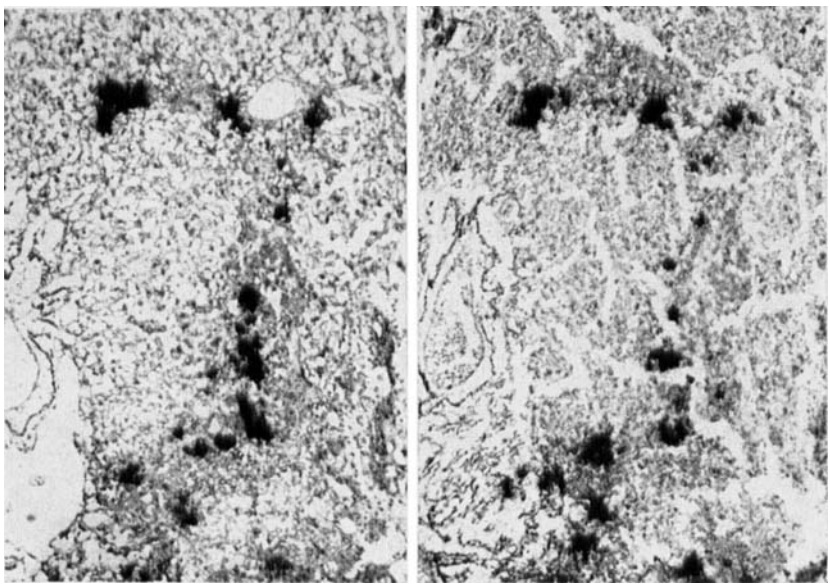


Fig. 6. Ca^{45} distribution in the same section as shown in Fig. 5. Uptake in foetal bones clearly visible.

The distribution of F^{18} and Ca^{45} 30 min. after peroral ingestion is demonstrated in Figs. 5 and 6. It appears that an exposure and photographic treatment of the emulsions that brings out the F^{18} uptake of the maternal skeleton at least as strongly as that of Ca^{45} hardly shows any F^{18} uptake by the foetal skeletons, while the



Figs. 7 and 8. F^{18} (left) and Ca^{45} (right) uptake in closely adjacent sections through full-term mouse placenta. The concentration of both isotopes to the same structures is evident. Photographs of stained 5 μ sections with adhering emulsions, about 65 $\times$.

Ca^{45} uptake in the latter is clearly visible. Nevertheless, the intestinal picture indicates a more complete absorption of F^{18} than of Ca^{45}.

The detailed autoradiograms of the placentae and foetuses thirty minutes after the injection showed that the small areas of concentrated F^{18}-activity were not to be found in all placentae. However, they were visible in many, and on consecutive sections one could locate the F^{18}- and Ca^{45}-uptake to the same tissue structure in the placentae and most often adjacent to the trophoblast layers (Figs. 7, 8).

No other foetal tissues than the bones and the teeth showed any F^{18}-uptake, and nothing indicated any paraplacental transfer of fluoride. The uptake by calcifying foetal tissues showed a similar picture as was found earlier in rat sucklings (*Ericsson, Ullberg & Appelgren* 1960).

QUANTITATIVE STUDY

Material and methods

F^{18} was produced and purified in the same way as described above, except that no concentration by evaporation of the distillate was performed.

$Ca^{45}Cl_2$ (spec. act. about 500 mc/g Ca) was obtained from the same source as given above and was diluted with saline before mixing with the F^{18}-solution for injection or ingestion.

Full-term pregnant white mice, fasting since about 18 hours, were used for the experiments. Four mice were each given an intravenous injection of 0.3 ml of the isotope mixture containing about 7 μC F^{18} and about 60 μC Ca^{45}. Eight mice were given each 0.6 ml of the same isotope mixture, diluted with water 1+1, by stomach syringe.

The intravenously injected animals were sacrificed 15 or 60 min., respectively, after the injection, while the survival time following peroral ingestion was 1 hour for 4 animals and 4 hours for the other 4.

Under trichlorethylene anaesthesia the animal was opened and blood was drawn from the heart with a heparinized syringe. Three foetuses and three placentae were taken from each mouse. From the animals that had obtained peroral doses the stomachs and intestines with their contents were also removed.

Every sample was placed in a weighed plastic counting tube fitting into the well of a scintillation crystal. After weighing, water was added to the same level in all tubes in order to give a standardized geometry, and the samples were counted on the same day and again after 2 days. It had been ascertained in advance that mixing with biological material (mincemeat and calcium phosphate) did not influence the scintillation counts measurably.

The second count (Ca^{45}) was deducted from the first one (Ca^{45}+F^{18}), and the resulting F^{18}-counts were recalculated to a standard time. The F^{18}- and Ca^{45}-contents of the various specimens could thus be calculated as percentages of the given doses per gram or per total organ.

Results

The results are given in Table 1 and in the main illustrated in Fig. 9.

It is seen that the foetuses took up a several times greater percentage of the Ca^{45}-doses than of the simultaneously administered F^{18}-doses, in spite of the much greater percentage of the Ca^{45}-dose found in the digestive tract after peroral ingestion. The rise of the foetal Ca^{45}-content is also much steeper during the experimental period than the corresponding rise of F^{18}-content. In spite of the presence of the rapidly mineralizing and Ca^{45}-accumulating skeletons of the foetuses there is significantly less F^{18} per gram foetus than per gram placenta in all animals sacrificed after 15 and 60 minutes while this ratio is reversed after 4 hours.

Table 1

F^{18} and Ca^{45} in blood, placenta, and foetuses of mice after intravenous and peroral administration, respectively, and in digestive tract after peroral administration.

Administration	F^{18}, % of dose				Ca^{45}, % of dose			
Survival time	Blood	Plac.	Foetus	Dig. tract	Blood	Plac.	Foetus	Dig. tract
Intravenous (individual values)								
¼ h.	1.97	1.39	0.35	—	4.00	2.50	1.39	—
	1.11	1.24	0.23	—	5.84	3.19	1.19	—
1 h.	0.30	0.58	0.45	—	5.84	1.82	2.69	—
	0.42	0.57	0.45	—	1.86	2.86	2.65	—
Peroral (averages ± stand. errors)								
1 h.	0.42	0.65	0.28	15.35	0.95	1.49	1.10	43.26
	±0.07	±0.11	±0.03	±3.60	±0.07	±0.04	±0.12	±13.32
4 h.	0.11	0.26	0.50	13.21	0.73	1.32	3.87	32.30
	±0.02	±0.07	±0.03	±7.22	±0.14	±0.11	±0.21	±10.75

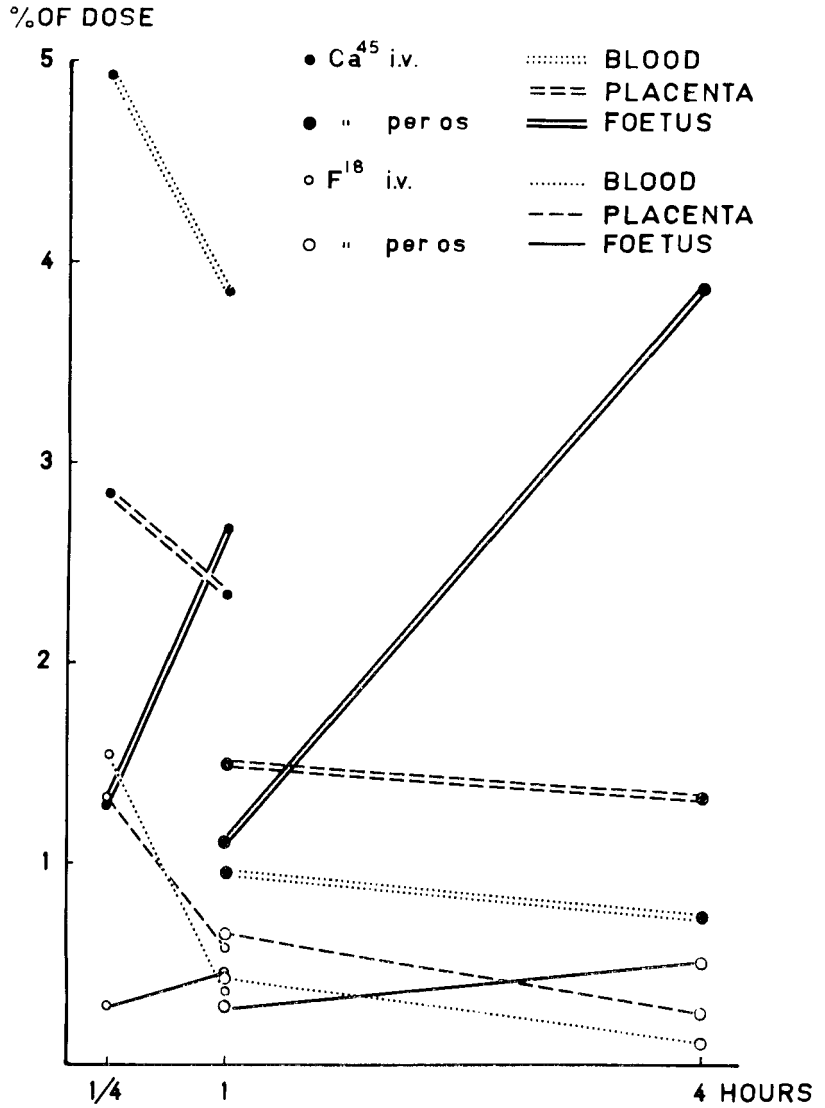


Fig. 9. F^{18} and Ca^{45} in blood, placenta, and foetuses after intravenous and peroral administration, respectively (illustrating Table 1).

DISCUSSION

Similarly as demonstrated by *Ericsson & Malmnäs* (loc. cit.) for the human and the rabbit, *Bawden & al.* (1964) reported very low foetal plasma F^{18}-levels in sheep following injection of the tracer into the ewe. These authors also reported the appearance in the maternal blood of F^{18} injected into the foetal circulation. It might be thought that the low F^{18}-content of the foetal blood compared to the maternal blood could be due to an extremely rapid clearance of the foetal plasma, especially during the mineralization period of the foetal skeleton. However, our autoradiograms and quantitative data demonstrate that this cannot be the case since the foetal skeletal uptake of F^{18} is much lower than that of the maternal skeleton and much lower than the corresponding uptake of Ca^{45}.

The slow diffusion through the placentae is liable, especially, to cut the passage of any transitional fluoride wave in the maternal blood, such as that simulated by the injection of radioactive fluorine in human mid-pregnancy, and in the late pregnancy of mice as showed by this investigation. Following an injection most of the placental fluorine content is evidently transported to the maternal hard tissues and kidneys by the maternal blood stream.

It is conceivable that the slow placental transfer of fluorine, when combined with the great homeostatic capacity for fluorine of the mammalian body (*Carlson & al.* 1960 a, b), should result in the low fluoride contents of the bones and teeth of newborn that have repeatedly been found. One of the clinical consequences of the low fluoride content of the deciduous teeth calcified *in utero* has long been known, viz. the rare occurrence of fluorotic mottling of these teeth. Another consequence seems to be the low protection of these teeth against caries by fluoride ingested by the mother, as reported in animal studies (*Büttner & Muhler,* 1958) as well as from a study in a fluoridation area (*Carlos & al.,* 1962). A consequence of this is, in turn, that there is little or no rationale in the fluoride ingestion often recommended to pregnant women for the protection of the expected child's teeth.

Concerning the small areas in the placentae that showed a concentrated uptake of F^{18}, there is now stronger evidence for their

being degenerative calcifications. In the present investigation they were not apparent in all placentae but, when present, they were found to coincide with areas and spots that had taken up Ca^{45}. Similar spots in the placentae can also be observed in the autoradiograms of some other bone-seeking trace elements such as P^{33} and Y^{91} (*Ullberg* 1964).

Such areas of calcification are probably responsible for the high fluorine content in the human placenta at the time of delivery that has been reported (*Gardner & al.* 1952, *Feltman & Kosel* 1961, *Borris* 1963). The observation in the present investigation that the calcium salt deposits are adjacent to the trophoblastic layers, is in agreement with reports by previous authors (*Dempsey & Wislocki* 1944).

The reports that the child, at birth, has about the same blood fluoride concentration as the mother (*Held* 1952, *Borris* 1963) may appear difficult to reconcile with the data and interpretations given above. However, also in adults with widely different supply and storage of fluoride the plasma fluoride concentration shows but small variations (*Armstrong & Singer* 1959).

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SUMMARY

The placental transfer of F^{18} as fluoride ions has been visualized autoradiographically in mice up to one hour following intravenous injection of the isotope. The foetal skeleton is seen to accumulate much less F^{18} than the maternal skeleton.

Following the simultaneous ingestion of $F^{18}+Ca^{45}$ an autoradiographic technique, which gives roughly similar uptake-pictures

for both isotopes in the maternal skeleton, has produced uncomparably stronger Ca^{45}-pictures of the foetal skeletons.

The often occurring spotty concentrations of F^{18} in the full-term mouse placenta were found to coincide with the concentrations of simultaneously injected Ca^{45}, and to relate to the general areas of the degenerative placental calcifications.

A quantitative study of the placental transfer in mice of simultaneously administered F^{18} and Ca^{45} showed a much smaller percentage of the F^{18}-dose than of the Ca^{45}-dose in the foetuses up to 4 hours, in spite of a more complete absorption of ingested F^{18}.

The results are discussed *i.al.* from a clinical point of view and are viewed in relation to clinical data on record in the literature.

RÉSUMÉ

PASSAGE DE F^{18} PAR LE PLACENTA DE LA SOURIS, EN COMPARAISON DU PASSAGE DE Ca^{45}

Le passage de F^{18}, comme ion fluorure, par le placenta de la souris a été démontré par autoradiographie jusqu'à une heure après injection intraveineuse de l'isotope. Le squelette foetal absorbe beaucoup moins du F^{18} que le squelette maternel.

Après ingestion simultanée de $F^{18}+Ca^{45}$ on trouve, avec une technique autoradiographique qui produit environ le même noircissement par les deux isotopes absorbés dans le sequelette maternel, que les images de Ca^{45} dans les squelettes foetaux sont beaucoup plus fortes.

Les concentrations de F^{18} par endroits dans le placenta à terme, préalablement décrites, coïncident avec les concentrations de Ca^{45} simultanément injecté et se localisent en général dans les zones de concrétions dégénératives.

Une étude quantitative du passage par le placenta de F^{18} et de Ca^{45}, simultanément administrés, a démontré un pourcentage de la dose de F^{18} très inférieur à celle de Ca^{45} dans les foetus jusqu'à 4 heures après l'administration en dépit de la résorption plus complète de F^{18}.

Les résultats sont discutés du point de vue clinique, entre autres.

ZUSAMMENFASSUNG

PLACENTAÜBERTRITT VON F^{18} BEI DER MAUS, MIT DEM ÜBERTRITT VON Ca^{45} VERGLICHEN

Der Placentaübertritt von F^{18} als Fluoridion ist bei der Maus autoradiographisch dargestellt worden, und zwar bis zu einer Stunde nach intravenöser Injektion des Isotopes. Man sieht eine viel grössere F^{18}-Aufnahme im Skelett des Muttertieres als im Fötalskelett.

Doppel-Tracer-Autoradiographie wurde nach gleichzeitiger peroraler Zufuhr von F^{18} und Ca^{45} gemäss einer Technik durchgeführt, die etwa das gleiche Schwärzen im Skelettbild des Muttertieres durch beide Isotopen bewirkte. Viel stärkere Bilder von Ca^{45} als von F^{18} sind dabei durch die Fötalskeletten erzeugt worden.

Die F^{18}-Konzentrationen, die in der reifen Mausplacenta stellenweise oft gefunden werden, fielen mit den Konzentrationen von gleichzeitig injiziertem Ca^{45} zusammen und zwar in den Gewebsteilen, wo degenerative Verkalkungen am häufigsten auftreten.

Der Placentaübertritt gleichzeitig zugeführter F^{18} und Ca^{45} ist auch quantitativ an Mäusen studiert worden. Bis zu 4 Stunden nach der Zufuhr ist ein viel niedriger Prozentgehalt der F^{18}-Dose als der Ca^{45}-Dose in den Föten gefunden worden, obgleich das F^{18} viel schneller aus dem Darm resorbiert worden ist.

Die Ergebnisse werden u. a. vom Gesichtspunkt einschlägiger klinischer Daten diskutiert.

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