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THE INCORPORATION OF ORALLY ADMINISTERED RADIOIODINE INTO HENS' EGGS AND FOLLICLES*)

By

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It is now generally accepted that of the food products which are contaminated by radioiodine in connection with reactor accidents and atomic explosions, milk and then eggs are the most important from the viewpoint of food hygiene. The hen's secretion of radioiodine by way of eggs has been studied by, among others, *Roche et al.* (1951), *Blanquet et al.* (1957), *Roche et al.* (1957) and *Okonski et al.* (1961).

In this work, the secretion of I^{131} by way of eggs with reference to the time after the administration has been studied under Swedish conditions in the country's most common chicken breed, the White Leghorn. The distribution and concentration of radioiodine in the yolk, the white and the shell has been followed partly after single administrations and partly following multiple administrations. Calculation of the concentrations found in the egg yolks with single dosing up to multiple dose concentrations has been done in order to see if the values obtained in such a manner agree with those obtained in the multiple dose experiments. In order to see how the amount of the follicles' uptake of radioiodine varies with the size of the follicle at the time of administration, the hens were killed at various times after

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administration and the concentration in the follicles analysed. Localisation of the radioiodine in the follicles and eggs has been investigated autoradiographically.

MATERIAL AND METHODS

Twenty laying hens of the White Leghorn Breed, which varied in age between 8 months and 2 years, were used for the investigation. The hens were kept in separate screen coops and had free access to oats, laying pellets, limestone and water. The feed consumption per hen per day varied between 50 and 100 g each of oats and pellets. Carrier-free I^{131} as sodium iodide in water solution in the amount of two ml was given orally to the hens by means of a stomach tube to the crop. The amount of I^{131} for the single doses varied between 10 and 50 μC. The original activity of the first dose of the multiple dose series was 10 μC. The dosing in the latter experiments was done once daily for 16 days. For the autoradiographic investigations, one mC of I^{131} was given as a single dose.

Measurements of radioactivity on eggs and follicles were done with a well-scintillation detector coupled to a single channel gamma-ray spectrometer. For judging the concentration in eggs and follicles, a prepared standard sample was used, one for each experiment. On this basis, the values were corrected for the physical half-life of I^{131}. For autoradiography of egg follicles *in situ,* the hens were euthanized with ether inhaltion. The defeathered body was rapidly frozen by immersion in n-Hexan cooled to about —70° with solid carbon dioxide. The freezing of the eggs was done in the same manner. Apposition autoradiograms were made on 100 μ thick sections using Structurix, Gaevert film. The exposure time was three days. The autoradiographic technique was that described by *Ullberg* (1958).*)

RESULTS

With single dose administration of I^{131}, the shell, including the shell membranes, showed the highest concentration in the egg laid within the first day after administration. The concentration was about 0.017 % of the given dose per g of shell. The

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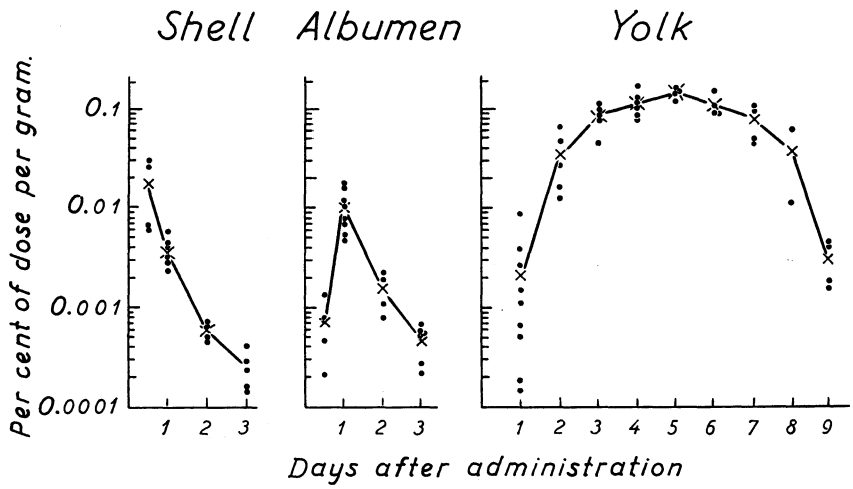


Figure 1. The concentration of I^{131} in shell, white and yolk in relation to the time after single dose oral administration to hens.
• Concentration in a single egg. × Mean value of the concentration.

shells' average weight was 7.8 g. The concentration in the shell then diminished rapidly and was about 1/70 of the maximum value three days after administration (Figure 1). With the continuous daily administration of I^{131}, an equally high concentration was shown rapidly in the shell. It then remained relatively constant and at the same level as the maximum concentration in the single dose experiment during the time interval that the dose lasted. Following cessation of administration, a rapid reduction of the concentration occurred in the shell similar to that with single dose administration.

The concentration of radioiodine in the white was maximal in egg laid about one day after single dose administrations and was about 0.01 % of the given dose per g of white. The average weight of the whites was 29.5 g. In the eggs which were laid later, the whites contained rapidly diminishing amounts of the radioiodine. Hence, the concentration three days after administration was hardly 1/20 of the maximum value (Figure 1). With daily dosing the white showed the same rapid concentration increase in the beginning of the experiments as in single dose experiments. The concentration remained relatively constant in the same range as the maximum value in single dose administration as long as the administration continued. Following cessation of

dosing the isotope showed a reduction in concentration similar to that seen in single dose experiments.

The concentration of I^{131} in the yolks following single dose administrations showed a course in accordance with Figure 1. The yolks from the eggs laid earlier than one day after administration showed no radioiodine content. The maximum concentration was found in yolks from eggs laid between four and six days after administration. In hens with low laying intensity the maximum concentration appeared in eggs laid a few days later than those mentioned above. Independent of laying intensity, however, maximal concentration in the yolk, as a rule, appeared in the fourth egg laid after administration. A reduction in the concentration in the yolks in relation to the time following administration appeared after the above mentioned time so that the concentration nine days after administration was about 1/50 of the maximal concentration (Table I).

Table I. The concentration of I^{131} in the yolk in relation to the time after single dose administrations.

Time after administration	Number of eggs	Per cent of given dose per g of yolk		Per cent of given dose per yolk
		Mean	Range	Mean
1 day	9	0.002	0.0002—0.009	0.04
2 days	5	0.035	0.013—0.068	0.65
3 „	5	0.087	0.047—0.116	1.62
4 „	6	0.117	0.082—0.175	2.19
5 „	4	0.148	0.126—0.167	2.78
6 „	4	0.115	0.095—0.159	2.15
7 „	4	0.080	0.046—0.114	1.50
8 „	2	0.039	0.012—0.066	0.73
9 „	4	0.003	0.002—0.004	0.06

Since the weights of the yolks varied between 14.7 and 24.2 g, the mean beeing 17.8 g, the amount of radioiodine per yolk expressed as per cent of given dose is stated in the table. This makes the comparison with the uptake in the follicles easier. During the continuous daily administrations of I^{131} increased concentration in the yolk was obtained from the eggs laid up to eight to ten days after the beginning of the administration. Thereafter, the concentration was relatively constant so long as the daily administration continued. An obvious reduction in the

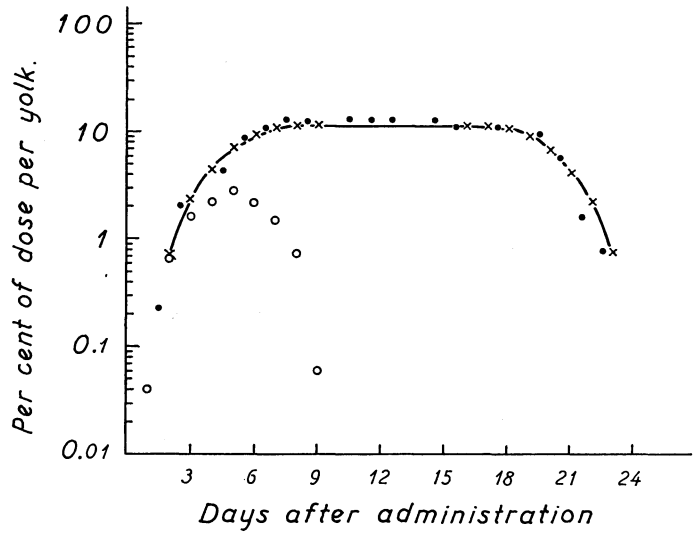


Figure 2. The amount of I^{131} per yolk in per cent of the daily dose in hens receiving daily doses for 16 days and the corresponding, theoretical values calculated from the mean values of the single dose administrations.

o Mean values after single doses. • Mean values after multiple doses. × The single dose values extrapolated into multiple dose values.

concentration in the yolk was first obtained in eggs laid five days after discontinuation of the dose. The concentration in the yolks laid seven days after discontinuation was about 1/20 of the equilibrium. The amount of I^{131} per yolk, expressed as per cent of the daily given dose, varied between 11.0 and 15.7 % (mean 12.8 %) in eggs laid between 9 and 17 days after commencement of the dosing. In Figure 2, the amount of radioiodine per yolk in the continuous administration is stated as well as the mean values from Table I for the single dose administrations. The latter have, in addition, been calculated to be valid for continuous daily administration. In this manner a theoretical equilibrium of 11.7 % of the daily dose per yolk was obtained. With the guidance of the concentration in the yolk, white and shell with the multiple dose experiments it was calculated that the entire egg contained about 13.2 % of the daily dose at equilibrium. A corresponding theoretical value was found to be 12.1 %.

In the investigation on follicles, follicles which contained a yellow yolk and which had a weight of at least 0.5 g were included. The follicles' uptake of radioiodine varied with the size. Depend-

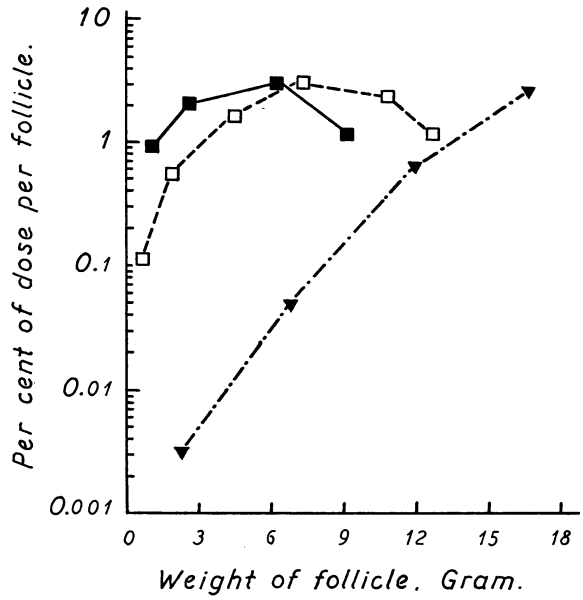
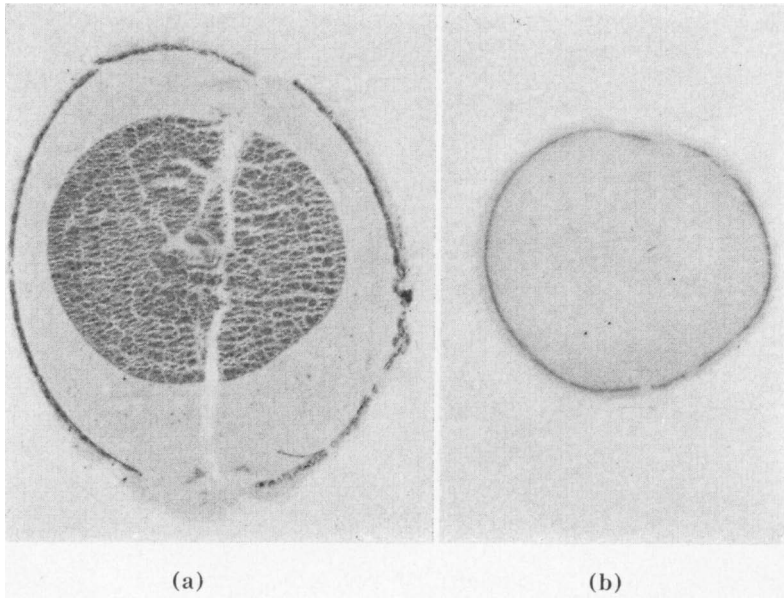


Figure 3. The amount of I^{131} in follicles expressed as per cent of the given single dose administration to hens with consideration of follicular weight and time between administration and slaughter.

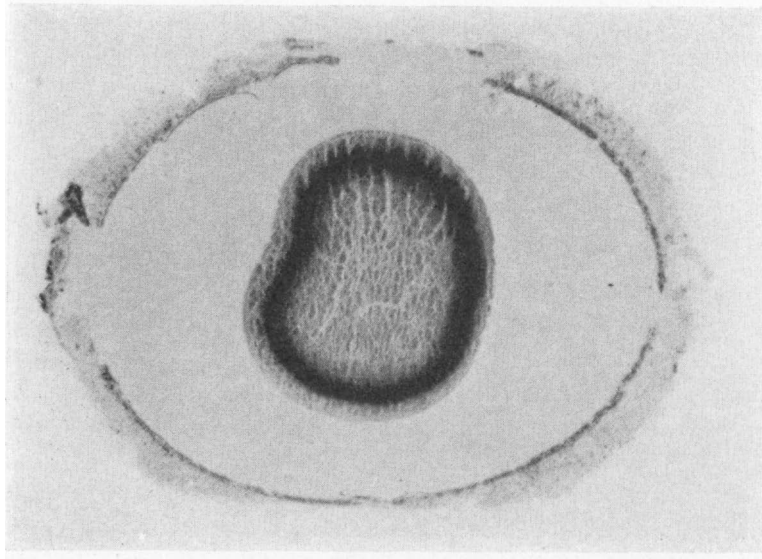
■ Hen killed one day after administration.
□ „ „ two days „ „ .
▼ „ „ four days „ „ .

ing upon the time interval between administration and sacrifice of the hen, and the follicular growth during this interval, the weight of that follicle which had the highest amount of radioiodine increased according to increasing length of the interval. This is seen in Figure 3. In nine hens killed between six hours and seven days after a single dose administration between 4.2 % and 10.6 % (mean 7.7 %) of the given dose was recovered totally in the yolks and follicles. In comparison, it can be reported that after single dose administration to four hens, a total of 7.3 % to 8.8 % (mean 8.2 %) of the given dose was rocovered in yolks from eggs laid during the subsequent eleven days. Statistically, no significant difference between the values in these two experiments was obtained.

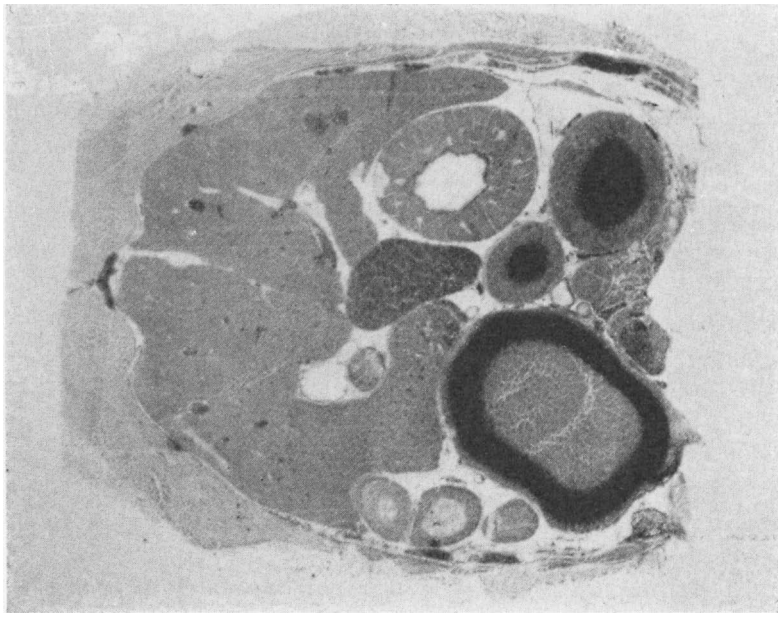
The autoradiographic investigation on the uptake of radioiodine following single dose administration showed that the uptake occurred rapidly and that the radioiodine did not blend itself evenly throughout the yolk. It lay in a sphere in the follicle



Picture I. Section of an egg laid 30 hours after a single oral administration of I^{131} to the hen (a) with autoradiogram of the section (b). The black ring on (b) indicates the site of I^{131} and corresponds to the outermost layer of the yolk in (a).



Picture II. Autoradiogram of a section from an egg laid 78 hours after a single oral administration of I^{131} to the hen. The picture was taken with the developed autoradiographic film placed over the section. The black ring in the yolk indicates the site of I^{131}.



Picture III. Autoradiogram of a transverse section from a hen at the level of the ovary. The hen was killed 72 hours after administration of a single oral dose of I^{131}. The picture was taken with the developed autoradiographic film placed over the section. The black field in three follicles indicates the site of I^{131}.

and later in the yolk in the laid egg. This sphere corresponded to the follicle's phase of growth during administration (Pictures I, II and III).

DISCUSSION

The time interval between rupture of a follicle and laying of a fully developed egg is about one day. During this time, the white and shell are formed. Most of this time is spent in developing the shell (*Romanoff & Romanoff* 1949). With the oral administration of I^{131} to hens, a rapid uptake in the blood occurs with maximal concentration within a few hours after the administration (own unpublished observation). Since the hen secretes a part of the given radioiodine by way of the white, the white which is formed when the blood concentration is highest should contain the highest concentration of radioiodine. An egg with this white is as a rule first laid about one day after the administration. If,

instead, shell formation occurs when the blood concentration is the highest, the shell will then contain a high concentration of radioiodine. Such an egg is generally laid earlier than one day after administration. This explains why, in the present investigation, in the single dose administrations, the maximal concentration of I^{131} is found in the shell in eggs laid about one half day after administration while maximal concentration in the white is found in egg laid about one day after administration. In spite of this, radioiodine appears in the white of eggs laid about one half day after administration. This may be explained by the fact that the white takes up some water with the salts therein when the true formation of the white is ceased and the shell formation is going on (*Romanoff & Romanoff* 1949). The same authors state also that the yolk, after the follicular rupture, absorbs some liquid on entering the oviduct and during the formation of the white. Timewise, this is a short period which explains why the radioiodine appears in the yolk first in eggs laid about one day after administration of the isotope.

Okonski et al. (1961) found, in hens with a single dose oral administration of I^{131}, maximal concentration in the shell and white of eggs laid one day after administration with a concentration between 0.003 % and 0.004 % of the given dose per g, both for shell and white. With long term ingestion the same authors found that the concentration in shell and white during equilibrium was about 0.01 % of the daily given dose per g. This later value agrees with the results of the present investigation. *Roche et al.* (1951) found that maximal concentration in the white after an intramuscular injection of I^{131} came two days after administration.

After single dose administration of I^{131} a considerable amount of the radioiodine in the eggs was found in the yolk with a maximal concentration of about 0.15 % of the given dose per g yolk in eggs laid about five days after administration. *Roche et al.* (1951), in one experiment, found maximal concentration in the yolk eight days after injection, and in another after five days. *Okonski et al.* (1961) found the maximum six days after administration with a concentration per g of yolk of about 0.1 % of the given dose. The variations in the time for maximal concentration may be dependent upon the hens' laying intensity. A low egg-laying frequency may displace the maximum, timewise, one or a few days.

Determinations of the amount of radioiodine in follicles after single dose administration showed that the highest amount occurred as a rule in the follicle which weight-wise was number four in the size arrangement (including eventually ruptured follicle and laid egg). The weight of the follicle with the largest content of radioiodine varied firstly with the time which passed between administration and killing of the hen. Large variations of weight of this follicle occurred also in hens killed with the same time interval. *Roche et al.* (1956) found with intramuscular administration of I^{131}, that the follicle in the third size classification had the largest amount of radioiodine in hens killed 12, 24 and 48 hours after administration. These follicles weighed then 11.2, 8.8 and 13.1 g, respectively. It is probable that the follicle which increases most rapidly after administration takes up the greatest amount of radioiodine. The fixed localisation of radioiodine in the follicles and then in the yolks as shown by autoradiographic studies is striking. This agrees with the investigations done by *Blanquet et al.* (1957). Following a single dose administration of I^{131} a total amount of about 8 % of the given dose was recovered in the follicles and yolks. This was independent of the time lapse between administration and slaughter. *Blanquet et al.* (1957) found, in follicles and eggs together, a maximum of about 20 % of the given subcutaneous dose four days after the injection. Their corresponding value two days after injection was about 10 %. *Roche et al.* (1957) found 11.7, 10.1 and 5.7 % of the given intravenous dose in the follicles 12, 24 and 48 hours, respectively, after administration.

With continuous daily doses of I^{131} to hen, in regard to the secretion by way of the yolks, an equilibrium was reached about nine days after the first administration. In one low-laying hen, this time was displaced a few days. About 13 % of the daily given dose per yolk was recovered during the equilibrium. *Okonski et al.* (1961) found the corresponding value to be 9 %. The difference here may be dependent upon, for example, feeding behovior or a variation in weight of the yolks. *Roche et al.* (1957) recovered a little more than 10 % of the intravenously given daily dose per yolk. For calculation of the concentrations found in the yolks with single dosing up to multiple dose concentrations the assumptions are made that the I^{131} once incorporated in the follicle is fixed and is recovered in the later laid yolk, and that in single dose experiments no apparent addition of I^{131} occurs during the

follicle's growth besides that which is incorporated during the first day after administration. A yolk laid nine days after a single dose administration thus has a content of radioiodine which is the same as it had as a little follicle. If instead, a new dose of I^{131} would be given to the hen one day after the first administration, this follicle would receive an additional amount of radioiodine corresponding to the amount in a yolk laid eight days after a single dose administration. With a third dose after one additional day the follicle would receive a new additional amount of radioiodine corresponding to the amount in a yolk laid seven days after a single dose administration. In the same manner, it may be assumed that during multiple dosing the follicle receives a daily supply of radioiodine corresponding to the amount which is recovered in corresponding laid yolk with the single dose administration. A calculation done in this manner using the values found in the present investigation from the single dose administration shows that theoretically in continuously daily dosing, every yolk from a laid egg should contain about 12 % of the daily given dose when equilibrium is reached. *Roche et al.* (1957) found in corresponding comparisons between single and multiple dosing that theoretically the yolk in an egg laid nine days after commencement of administration should contain a little over 10 % of the given daily dose while the investigation, experimentally carried out, showed 12 % of the daily dose in the yolk from an egg laid six days after commencement of administration. *Okonski et al.* (1961) found in their investigations, no agreement between the theoretically and experimentally obtained results.

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SUMMARY

Incorporation of radioiodine into eggs and follicles was studied by oral administration of I^{131} to hens.

With the single dose administration of I^{131} the shell, including the shell membranes, reached a maximal concentration of 0.017 % of the given amount per g of shell in eggs laid within one day after administration. The white reached a maximal concentration of 0.01 % per g in eggs laid about one day after administration. The subsequent reduction in concentration both in the shell and white from later laid eggs showed a rapid course. In the yolk, radioiodine began to appear in eggs laid about one day after administration. A maximal concentration of 2.78 % of the given amount per yolk was reached in eggs laid about five days after administration. The subsequent reduction in concentration could be followed in the yolk from eggs laid up to and including the ninth day after administration; then the concentration was about 1/50 of the maximal concentration.

With continuous administration of I^{131} once daily for 16 days, the concentration in the shell and white was in the same amount as corresponding maximal concentration in single dose administration as long as the daily supply continued. Following cessation of administration, the concentration decreased in the shell and white with a course simliar to that obtained in the single dose administration. In the yolk, the concentration increased in eggs laid up to and including the ninth day after commencement of the continuous dosing; thereafter, the equilibrium remained as long as the administration continued. About 13 % of the daily given dose of I^{131} was recovered per yolk. After discontinuation of administration, an obvious concentration reduction was first obtained in the yolks from eggs laid five days later. A calculation of the results for the yolks from the single dose administration to be valid for the daily continuous dosing showed good agreement with the results obtained in the present multiple dose experiments.

The follicular uptake of I^{131} in single dose administration was rapid, with the greatest uptake by the follicle which was in the most rapid phase of growth at the time of administration. During autoradiography of the follicles and eggs following single dose administration of I^{131}, most of the radioiodine was recovered in the growth zone which corresponded to the follicle's development during the first day after administration.

ZUSAMMENFASSUNG

Aufnahme von Radiojod in Hühnereiern und Follikeln bei Eingabe per os.

Die Aufnahme von Radiojod in Eiern und Follikeln wurde mittels Eingabe von J^{131} per os an Hühnern studiert.

Bei einmaliger Gabe von J^{131} erhielt man in der Schale, einschliesslich der Schalenhäute, eine maximale Konzentration von 0.017 % der gegebenen Menge per Gramm Schale von, innerhalb eines Tages nach Eingabe gelegten Eiern. Im Eiweiss erhielt man eine maximale Konzentration von 0.01 % der gegebenen Menge Radiojod per Gramm Eiweiss von Eiern, die ungefähr einen Tag nach Eingabe gelegt worden waren. Die darauffolgende Konzentrationsabnahme in sowohl Schale als auch Eiweiss von später gelegten Eiern, zeigte einen schnellen Verlauf an. Radiojod begann in der Dotter von Eiern, die ungefähr einen Tag nach Eingabe gelegt worden waren, autzutreten. Eine maximale Konzentration von 2.78 % der gegebenen Menge Radiojod per Dotter erhielt man von Eiern, die ungefähr fünf Tage nach Eingabe gelegt worden waren. Die darauffolgende Konzentrationsabnahme konnte man in, bis einschliesslich des neunten Tages nach Eingabe gelegten Eiern folgen, wobei die Konzentration ungefähr 1/50 der maximalen aufwies.

Bei kontinuierlicher Gabe, einmal täglich unter 16 Tagen, war die Konzentration in Schale und Eiweiss in der gleichen Grössenordnung, wie die entsprechende maximale Konzentration bei einmaliger Gabe, solange die tägliche Zufuhr anhielt. Nach abgeschlossener Eingabe nahme die Konzentration in Schale und Eiweiss mit einem gleichartigen Verlauf, wie der, der bei der einmaligen Gabe erhalten worden war, ab. In der Dotter nahm die Konzentration in Eiern, die bis einschliesslich des neunten Tages nach begonnener kontinuierliches Zufuhr gelegt worden waren, zu; darauf erhielt man ein Gleichgewicht, solange die Zufuhr anhielt. Ungefähr 13 % der täglich gegebenen Menge J^{131} fand man dabei per Dotter. Nach abgeschlossener Gabe erhielt man eine offenbare Konzentrationsabnahme in der Dotter nicht eher als in Eiern, die fünf Tage später gelegt worden waren. Berechnet man aus den Werten für Eigelb nach einmaliger Gabe den Gehalt für kontinuierliche Zufuhr, erhält man eine relativ gute Übereinstimmung mit den erhaltenen Werten des zum Schluss erwähnten Versuches.

Die Follikelaufnahme von J^{131} bei einmaliger Gabe war hastig und hatte die grösste Aufnahme in dem Follikel, der sich während der Eingabe in der schnellsten Zuwachsphase befand. Bei der Autoradiographie von Follikeln und Eiern nach einmaliger Gabe fand man den grössten Teil des Radiojodes in der Zuwachszone, der dem Zuwachs des Follikels während des ersten Tages nach der Eingabe entspricht.

SAMMANFATTNING

Inbyggnaden av radiojod i hönsägg och folliklar vid per oral ingivning.

Inbyggnaden av radiojod i ägg och folliklar studerades vid per oral ingivning av J^{131} åt höna.

Vid engångsingivning av J^{131} erhölls i skalen inklusive skalhinnor en maximal koncentrion av 0,017 % av given mängd per gram skal i ägg värpta inom ett dygn efter ingivningen. I vitan erhölls en maximal koncentration av 0,01 % av given mängd radiojod per gram vita i ägg värpta omkring ett dygn efter ingivningen. Den efterföljande koncentrationsminskningen både i skal och vita från senare värpta ägg visade ett snabbt förlopp. I gulan började radiojod uppträda i ägg värpta omkring ett dygn efter ingivningen. En maximal koncentration av 2,78 % av given mängd radiojod per gula erhölls i ägg värpta omkring fem dygn efter ingivningen. Den efterföljande koncentrationsminskningen kunde följas i gulan från ägg värpta till och med nio dygn efter ingivningen, då koncentrationen var omkring 1/50 av den maximala koncentrationen.

Vid kontinuerlig ingivning av J^{131} en gång dagligen i 16 dygn var koncentrationen i skal och vita i samma storleksordning som motsvarande maximala koncentration vid engångsgivor, så länge den dagliga tillförseln varade. Efter upphörd ingivning minskade koncentrationen i skal och vita med ett förlopp likartat det som erhölls vid engångsgivor. I gulor ökade koncentrationen i ägg värpta till och med det nionde dygnet efter påbörjad kontinuerlig tillförsel, varefter jämvikt erhölls så länge ingivningen varade. Omkring 13 % av daglig given mängd J^{131} återfanns då per gula. Efter avslutad ingivning erhölls en påtaglig koncentrationsminskning först i gulor från ägg värpta fem dygn senare. En kalkylering av värdena för gulorna vid engångsgiva till att gälla vid daglig kontinuerlig tillförsel, visar en relativt god överensstämmelse med de värden so merhölls vid sistnämnda försök.

Follikelupptaget av J^{131} vid engångsgivor var snabbt och med störst upptag av den follikel, som var i den snabbaste tillväxtfasen vid ingivningstillfället. Vid autoradiografi av folliklar och ägg efter engångsgiva återfanns den mesta radiojoden i den tillväxtzon som motsvarade follikelns tillväxt under första dygnet efter ingivningen.

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