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RESIDUES OF DRUGS IN EGGS AFTER MEDICATION OF LAYING HENS FOR EIGHT DAYS

By

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BLOM, LARS: *Residues of drugs in eggs after medication of laying hens for eight days.* Acta vet. scand. 1975, *16*, 396—404. — Drug residues in blood plasma, egg-white and -yolk have been measured for 3 weeks after 8 days of continuous treatment with sulphanilamide, sulphadimidine, sulphaquinoxaline and pyrimethamine. The results are discussed with reference to physiological data concerning egg-white and -yolk formation. From this, it is concluded that a withdrawal period of at least 10 days after the disappearance of drug in blood plasma is necessary to avoid residues of drugs in eggs from treated hens.

drug residue; sulphonamides; pyrimethamine; egg-white; egg-yolk.

Drug residues in eggs after medication of the laying hens have in the past been measured for a number of antibiotics and chemotherapeutics (a survey of the literature is given by *Blom & Rasmussen* 1973). As the result of these studies disappearance times (i.e. periods where drug-residues are detected) from a few days and up to 1 or 2 months after medication have been obtained.

A few authors (see DISCUSSION) have pointed out the very special physiological circumstances concerning the period of time in which the formation of egg-white and -yolk takes place and have stressed the importance of this with regard to the length of time in which drug residues are found in egg-white and -yolk.

In the present paper the residues of sulphanilamide, sulphadimidine, sulphaquinoxaline and pyrimethamine in egg-white and -yolk after medication of laying hens will be discussed in relation to the physiological findings concerning egg-white secretion and the process of egg-yolk formation.

MATERIALS AND METHODS

Sixty White Leghorn hens were divided into 5 groups each consisting of 12 hens. Group number 1 served as control group, while the groups numbers 2—5 were treated for 8 days by giving drinking water ad libitum containing 0.1 % sulphanilamide (Sulfanilamidum NFN), 0.1 % sulphadimidine (Sulfadimidinum NFN, Sulphamethazine), 0.04 % sulphaquinoxaline (Sulfabenzpyrazinum NFN) and 0.01 % pyrimethamine (Pyrimethaminum NFN), respectively.

During the experimental period and a period of 3 weeks after cessation of treatment eggs were collected and blood samples were taken daily. Details concerning the experimental procedure are given in a previous paper (*Blom* 1975).

Sulphonamide concentrations were measured according to *Bratton & Marshall* (1939) as described by *Blom* (1975) with a sensitivity of approx. 1 µg/ml or /g in the case of blood plasma and egg-white and 2 µg/g in the case of egg-yolk. For measuring smaller amounts of sulphonamides in egg-white and -yolk the method described by *Nielsen et al.* (1974) was used. This includes extraction with chloroform, evaporation until dryness, dissolving of the solid residue in hexane from which the sulphonamides are extracted by 1 N-HCl. The method has a sensitivity of 0.04 µg/g, when 25 g material is used for analysis.

The pyrimethamine content of blood plasma, egg-yolk and -white was measured according to *Schmidt et al.* (1953) modified by *Blom* (1975), with a sensitivity of 0.5—1.0 µg/ml or /g.

All eggs from group 2 were analyzed individually. From the groups 3—5, 3 eggs were analyzed individually each day, while the rest of the daily eggs were mixed making 1 sample of egg-yolk and 1 of egg-white for analysis.

All results listed in Tables 1—4 are given as daily mean of the group.

RESULTS

The concentrations of drugs in blood plasma, egg-white and -yolk at different times during the period of 3 weeks after 8 days of continuous treatment with sulphanilamide, sulphadimidine, sulphaquinoxaline and pyrimethamine are listed in Tables 1—4.

The results show residues of drugs in egg-white and -yolk several days after the drug no more could be detected in blood

Table 1. Average residual amount of total sulphanilamide in blood plasma, egg-white and -yolk after cessation of treatment.

Days after cessation of treatment	Blood plasma µg/ml**	Egg-white µg/g	Egg-yolk µg/g	Number of egg-white and -yolk samples
0	58	96	93	5
2	8	56	102	3
4	4	5	84	5
6	trace*	3*	45	8
8	trace	1	7	5
10	trace	0.7	3	6
12	0.0	0.2	1.6*	8
14	—	0.0	0.4	10
16	—	—	trace	6
18	—	—	0.0	11

* first day a drug-free sample was found.
** daily average of 6 samples.

Table 2. Average residual amount of total sulphadimidine in blood plasma, egg-white and -yolk after cessation of treatment.

Days after cessation of treatment	Blood plasma µg/ml**	Egg-white µg/g***	Egg-yolk µg/g***
0	41	52	51
2	13*	38	32
4	trace	1*	7
6	0.0	trace	0.4*
8	—	0.0	trace
10	—	—	0.0

* first day a drug-free sample was found.
** daily average of 6 samples.
*** daily average of 4 samples.

plasma, and in general the disappearance times are greatest in the case of the egg-yolk.

The disappearance times from blood plasma, egg-white and -yolk after cessation of treatment are given in Table 5. From this table it is seen that sulphadimidine has the shortest disappearance time from all media, while pyrimethamine has the longest and persists for more than 20 days in the egg-yolk.

Table 3. Average residual amount of total sulphaquinoxaline in blood plasma, egg-white and -yolk after cessation of treatment.

Days after cessation of treatment	Blood plasma µg/ml**	Egg-white µg/g***	Egg-yolk µg/g***
0	96	51	41
2	35	47	39
4	3*	5	36
6	trace	0.5*	10
8	0.0	trace	7
10	—	0.0	2*
12	—	—	trace
14	—	—	0.0

* first day a drug-free sample was found.
** daily average of 6 samples.
*** daily average of 4 samples.

Table 4. Average residual amount of pyrimethamine in blood plasma, egg-white and -yolk after cessation of treatment.

Days after cessation of treatment	Blood plasma µg/ml**	Egg-white µg/g***	Egg-yolk µg/g***
0	1.6	1.8	100
2	0.9	1.5	107
4	0.8*	0.8	79
6	trace	trace*	41
8	0.0	trace	24
10	—	0.0	4
12	—	—	4
14	—	—	3
16	—	—	2*
18	—	—	1
20	—	—	trace

* first day a drug-free sample was found.
** daily average of 6 samples.
*** daily average of 4 samples.

Table 5. Number of days after cessation of treatment where the drug was detected in the samples.

Drug	Blood plasma	Egg-white	Egg-yolk
Sulphanilamide	11	14	17
Sulphadimidine	5	7	10
Sulphaquinoxaline	8	10	13
Pyrimethamine	8	10	>20

DISCUSSION

The plasma half-lives for sulphanilamide, sulphadimidine, sulphaquinoxaline and pyrimethamine in the laying hen have previously been estimated to be 800, 1100, 1300 and 250 min., respectively (*Blom* 1975). The disappearance times from blood plasma in the case of sulphadimidine and sulphaquinoxaline are in agreement with their plasma half-lives. With regard to sulphanilamide and pyrimethamine, their disappearance times from blood plasma far exceeded those expected from their plasma half-lives. Both drugs have a high apparent volume of distribution in hens (V_d of 2.0 and 2.3, respectively, *Blom* 1975) which means that these drugs are present in higher concentrations in tissues than in blood plasma (*Butler* 1971). Pyrimethamine is highly lipid soluble (with a chloroform/water coefficient at pH 7.4 of 110, vide *Blom* 1975) and the long period in which pyrimethamine is detected in blood plasma can be explained by a slow passage of drug from adipose tissue to blood plasma.

After a similar treatment as in this study *Krieg & Siegmann* (1967) found a blood plasma disappearance time for sulphadimidine of 2 days, while *Lüders et al.* (1974) with an analytical sensitivity of 5 µg/ml found no sulphadimidine in blood plasma at post-treatment day 3.

The long blood plasma disappearance time for sulphaquinoxaline of 8 days (Table 3) is in agreement with *Righter et al.* (1973) who found 0.1 µg/ml (their analytical sensitivity level) sulphaquinoxaline in serum 10 days after cessation of the treatment with 0.1 % sulphaquinoxaline in the drinking water to turkey poults. With a sensitivity of 5 µg/ml, *Lüders et al.* could not detect any sulphaquinoxaline in the blood from 3 days after cessation of treatment with 0.05 % sulphaquinoxaline in the drinking water, while *Schlenker & Simmons* (1950) still found small amounts of sulphaquinoxaline in the blood 4 days after cessation of treatment with 0.0125 % in the feed.

Measurements of residues of drugs in the egg-white and -yolk have in all studies given longer disappearance times than in the case of blood plasma. Thus, *Krieg* (1966) detected no sulphadimidine in egg-yolk and -white day 8 and 6, respectively, after cessation of similar treatment as in this study. With an assumed sensitivity of 1 µg/g the results are in agreement with those obtained in this study (Table 2).

After treatment with 0.0125 % sulphaquinoxaline in the feed

Schlenker & Simmons found no drug in whole-egg at post-treatment day 8. *Righter et al.* (1970) have reported concentrations of 1.1 µg/g and 0.03 µg/g sulphaquinoxaline in egg-yolk and -white, respectively, 5 days after intermittent feeding with 0.05 % sulphaquinoxaline for 12 days.

However, the analytical sensitivity is of the greatest importance for the estimated length of the disappearance time. In a previous study (*Blom* 1974), the presence of sulphadimidine in the egg-yolk and -white was shown by spraying the reagents used for the analysis on the cut-surface of the boiled egg. By this method sulphadimidine residue was detected at post-treatment day 10 as a small red spot in the center of the egg-yolk, while no sulphadimidine was detected at that time by the laboratory analysis with a sensitivity of 0.04 µg/g egg-yolk. During the blending of the yolk before analysis the residual amount of drug is mixed with the total amount of egg-yolk, giving a final concentration, which is not detectable.

The lag period of approx. 1 day, between the disappearance of drug from blood plasma and that of egg-white (Table 5), was to be expected from physiological and morphological studies. Thus, *Oades & Brown* (1965) showed that the oviduct contains sufficient water-soluble proteins for about 2 eggs, and injection of radioactive aminoacids is followed by radioactivity in the egg-white 2 days later (*Siva-Sankar & Theis* 1959, *Mandeles & Ducay* 1962).

The drug concentration in yolk depends mainly on the lipid solubility of the drug (*Blom* 1975). The greater lipid solubility of a drug the greater concentration of the drug in the egg-yolk and a longer period with residues are obtained, as shown in this study with pyrimethamine.

Regarding the very long delay between the disappearance of the drug from blood plasma and that of egg-yolk, *Raica et al.* (1956), *Krieg* and *Sisodia & Dunlop* (1972) have pointed at the physiological facts concerning egg-yolk formation and especially the length of the third stage of egg-yolk development, where the egg-yolk grows from about 6 mm to 35 mm in diameter (*Marza & Marza* 1935).

This period of time has been estimated by *Hansen* (1928) feeding laying hens with daily doses of the lipid soluble dye Sudan III and measuring the period of time until the entire egg-yolk was coloured. *Riddle* (1908) and *Warren & Conrad* (1939),

using intravenous injection of Sudan III at 24 hrs. interval, counted the number of concentric red coloured rings obtained on the cut-surface of the egg-yolk. From these and later studies the length of the third growth phase have been estimated to between 7 and 11 days (*Gilbert* 1971). Similar results were obtained by *Blom* (1974) injecting a single intravenous dose of Food Green No. 1, Sudan III or Sudan Black to laying hens. After this treatment all eggs laid showed a distinct coloured ring on the cut-surface of the egg-yolk (but no colouring of the egg-white, similar to the results of *Denton* 1940) decreasing in diameter day by day until the tenth day after injection, where only a coloured spot in the center (the latebra) could be detected. Those findings showed that both water soluble (Food Green No. 1) and lipid soluble (Sudan III, Sudan Black) dyes were deposited in the egg-yolk, and the distinct coloured rings showed that very little diffusion if any takes place within the egg-yolk, supporting the statement of *Krieg* who concluded that once the sulphonamide is deposited in the egg-yolk it stays there.

The reason why some of the lag periods between the zero concentration in blood plasma and egg-yolk obtained in the present study have been shorter than the average 10 days one would expect from the period of egg-yolk development is probably due to the sensitivity of the analytical method used.

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SAMMENDRAG

Rester af lægemidler i æg efter 8 dages behandling af æglæggende høns.

Lægemiddelkoncentrationen i blodplasma, æggehvide og -blomme er bestemt i tre uger efter ophør af 8 dages kontinuerlig indgift af sulfanilamid, sulfadimidin, sulfabenzpyrazin og pyrimethamin til æglæggende høns. Disse resultater sammenholdes med de fysiologiske forhold, hvorunder æggehviden og -blommen dannes. På denne baggrund konkluderes, at tilbageholdelsestider på mindst 10 dage efter ikke målelige lægemiddelkoncentrationer i blodplasma må være et minimumskrav at stille til æg fra behandlede høns for at sikre konsumenterne mod rester af lægemidler i æg.

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