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Investigation of the role of selenium in the nutrition and physiology of neural tissues of chickens and turkeys.

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SUMMARY

Endogenous selenium concentrations in selected tissues of chicken, turkey and coturnix are presented. The selenium concentration for laying chicken organs and tissues were variable and in decreasing order as follows: pineal, pituitary, kidney, spleen, egg yolk, liver, pancreas, magnum, cerebrum, diencephalon, cerebellum, blood, ovary and pectoral muscle. The order for turkey organs was similar except that for pineal which was much lower and similar in concentration to the ovary. In coturnix the order was similar except the pineal had a very low concentration of selenium similar to the cerebellum and the pancreas had a high concentration exceeding that of the pituitary.

Distribution of tracer ^{75}Se in various chicken organs 30 minutes after injection found the greatest amounts in the liver and kidney, followed by pineal and by small amounts in the cerebellum. This ranking remained the same when the occurrence of ^{75}Se in tissues was expressed as the ratio to that of blood. Although species variations exist, selenium appears to concentrate in glandular organs (pineal, pituitary, pancreas), and in detoxifying and excreting organs (kidney, liver, spleen), which are generally characterized by some form of protein synthesis. A blood-brain barrier probably accounts for the general low levels of selenium found in diencephalon, cerebrum and cerebellum.

Selenium concentrations in chicken and coturnix eggs approximated that of the liver. Selenium in eyes of turkey and coturnix were low, similar to that reported in chickens.

The existence of extra-retinal photoreceptors in the brain has been suggested by numerous workers, e.g., Oishi et al., (1966) and Menaker (1968). However, microscopic examination of the pineal and of the brain reveal no pigmented photoreceptors. The suggestion that retinal selenium may have an important role as a photon receptor and energy transformer (Aberg, 1966) suggested to us that other neural areas may be high in selenium. Present technological application of the element in photocells appear to involve only the elemental forms, and it was thought that a general survey of tissues for selenium concentrations without regard to oxidation-reduction status might provide a starting point for an investigation of a possible role of the element in certain aspects of avian physiology. This is a report on the relative distribution of selenium in not only the pineal but also in other avian tissues.

Although toxic aspects of excess selenium in animal diets have been the subject of earlier researches, evidence in the past quarter century has been accummulating for a definite nutritional role of the element in vertebrates. As early as 1941, Poley et al., reported a growth response from feeding a diet containing 2 p.p.m. Se to chicks.

Implication of selenium in muscular dystrophy and exudative diathesis and fertility in chickens, as well as in a muscular myopathy (white muscle disease) of calves and lambs, is discussed in detail in Selenium in Biomedicine (Muth, 1967) and by Scott (1966), Savage (1968), Wright and Mraz (1964) and Jensen (1968). For a comprehensive review of selenium, readers are referred to Rosenfeld and Beath (1964).

This report summarizes analytical results for endogenous selenium distribution in selected tissues of chickens, turkey and coturnix (Japanese Quail), as well as tracer distribution of injected (intravenously) ^{75}Se.

PROCEDURE

Experiments designed to study endogenous selenium concentrations in chickens and also ^{75}Se distributions were carried out in one year old white Leghorn males (Expt. 1) and 24 week old white Leghorn males (Expt. 2). All birds were maintained under uniform temperature and light regimens for two weeks prior to death. They received chicken breeder pellets containing 3.42 nanogram atoms Se per gram (0.27 p.p.m.) for their life span.

In experiments 1 and 2, both groups of birds were killed at the first and second half of both the light and dark periods. The purpose was to avoid any diurnal rhythm that may be affecting the Se content of tissues. Thirty minutes before death each bird was injected with 400 microcuries of ^{75}Se as H_2SeO_3 in 0.2 ml. normal saline in experiment 1 and 200 μCi. in experiment 2. The H_2SeO_3 provided only 46 ng. Se per bird, which represented an insignificant increment of the element to the endogenous Se of the bird. At death, blood samples and tissues were obtained and transferred to vials for counting of ^{75}Se activity and for later analysis for total selenium. Radioactivity was measured in a deep well gamma counter. Total selenium was determined by ashing and fluorometry as described by Watkinson (1966).

The results of experiments 1 and 2 with Leghorn males indicated a need to undertake a comprehensive survey of selenium distribution in tissues of additional species, both male and female. For the purpose

chickens, turkeys and coturnix were reared and maintained under standard conditions, and the Se concentration of various tissues and organs measured (Expt. 3). Additional turkey and coturnix were killed in experiment 4 to provide more samples of pineal, pituitary and eye for analysis.

Tissue samples taken from each bird at death were weighed and stored on dry ice until analyzed. Samples of blood, cerebellum, cerebrum, diencephalon, kidney, liver, spleen, ovary, magnum, pancreas, pectoral muscle, feces and crop content weighing approximately 300 mg. were taken from each bird. Because of the small size (about 5 mg.) several samples of pineals and pituitaries were pooled to provide a sufficient amount of tissue for analysis. Samples of the feed and water were analyzed also for Se content. Available eggs were separated into shell, yolk and albumen for Se analysis. The entire eyes of turkeys and coturnix were analyzed without separation into parts. The turkey and coturnix diet contained 4.25 nanogram-atoms Se per gram (= 0.35 p.p.m.) and the chicken diet contained 3.42 nanogram-atoms Se per gram (= 0.27 p.p.m. Se). The drinking water had 5.0 nanogram-atoms Se/liter (= 0.0004 p.p.m. Se).

Statistical treatment consisted of analysis of variance and Scheffe's test (1953).

RESULTS AND DISCUSSION

Endogenous selenium concentration:

A considerable variation was found in the concentration of endogenous selenium in the tissues of male chickens one year old (Table 1a) versus 24 weeks old (Table 2a), however the trend of the data was similar.

In both trials selenium concentration was much higher in the pineal than in blood or other tissues. Absolute concentration rankings varied between experiments as follows: pineal > kidney > blood > liver > cerebellum (Expt. 1), and pineal > kidney > liver > blood > cerebellum (Expt. 2). The reversal of order of ranking between liver and kidney in the two experiments is not considered significant.

The distribution ratio (D) of endogenous tissue selenium concentration to endogenous blood selenium concentration ($D = \frac{\text{tissue Se}}{\text{blood Se}}$) again ranks the pineal significantly higher than other tissues studied in both experiments. The high concentration in the excretary organs agrees with Hartley (Muth, 1967). The very low ratio for cerebellum ($D < 1$) suggests an effective blood-brain barrier.

The localization of ^{75}Se in the tissues following injection of tracer amounts was rather uniform in both experiments (Tables 1b and 2b). The concentration in the liver and kidney was significantly higher than in blood, pineal or cerebellum. These results are in agreement with the results of Wright and Bell (1964) with sheep and Wright and Mraz (1964) with chickens, but they differ in some detail from results reported by Jensen et al. (1963), with young chicks. The latter found the highest values in blood, followed in order by the spleen, kidney and liver. The role of the liver and kidney may be associated with either metabolism and/or elimination of selenium. The distribution ratio of tracer amounts of ^{75}Se was again less than one for the cerebellum, indicating that this neural tissue is not a select area of localization, in contrast to the pineal.

The results of a more extensive survey (Expt. 3) of endogenous selenium concentrations in a variety of organs and tissue of turkeys and chickens

and a limited number (Expt. 4) of organs of coturnix and presented in Tables 3 and 4, respectively. The spleen, pancreas, and ovary of all three species had a higher selenium concentration than did the blood, but was less than that of pituitary, kidney and liver. Other organs and tissues contained similar concentrations of selenium to that of blood. The Se content of the pineal was higher than blood in turkeys and chicken but not in coturnix. These comparisons are somewhat misleading for the dry matter percentage of tissues are higher than those of blood.

Selenium concentration of crop contents and feces were similar for turkey, 1.6 and 1.6 nanogram-atoms Se/gm., respectively, and 0.9 and 1.0 nanogram-atoms Se/gm. for chicken. These concentrations bear a direct relation to the selenium content of the feed and water.

A limited number of analysis of eggs of chickens and quails were done (Table 3). In each case the yolk had the highest selenium concentration, with that of albumen and shell insignificantly lower. Taussky et al. (1963) reported values (recalculated by us) to be 5.13 nanogram-atoms Se per gram fresh egg yolk and 1.27 nanogram-atoms Se per gram for albumen. Our values of 6.7 and 7.9 for yolk and 1.3 and 1.8 for albumen are in good agreement with theirs. Hadjimarkos and Bonhorst (1964) reported the selenium contents of yolk and albumen to be in the ratio of 6.3 to 1. Our data gives a ratio of 5.1 to 1 for chicken egg and 4.65 to 1 for quail eggs. Using neutron activation for dried egg yolk, McConnell and Wabnitz (1964) obtained a concentration value (recalculated by us) of 15.9 nanogram-atoms Se per gram dry matter. If we assume that the moisture content of egg yolk is 48%, we calculated this to be 8.3 nanogram-atoms Se per gram of fresh yolk as compared to

our fresh matter basis values of 6.7 for chicken and 7.9 for coturnix egg yolk.

The concentration of selenium in the blood of laying chickens (Table 3) was considerably lower than that obtained in male chickens (Table 1a and 2a). Whether this reflects a true sexual difference or a physiological drain related to egg production or other factors requires further study. The concentration of selenium was greatest in the blood of coturnix, as well as the reproductive organs of this species.

Selenium concentration in the pineal (Tables 1, 2, 3 and 4) was highly variable among species, and its endogenous concentration was consistently the highest value of all tissues examined in the chicken (Tables 1a, 2a, and 3). The laying chicken had the lowest concentration and males the highest. This may suggest that selenium in the pineal is highly labile and perhaps subject to flux resulting from environmental or other variables not controlled in these studies.

Pituitary selenium concentrations were high in all 3 species (Tables 3 and 4), and did not vary among 5 sets of data obtained from the 3 species.

Selenium concentrations of whole eyes (right) of turkey and coturnix are presented in Table 4. Concentration of selenium in the eye of coturnix was greater than that of the turkey. For comparison, the value of 0.19 mg. per gram fresh eye for chickens reported by Taussky et al. (1966) recalculated to our units gives a concentration of 1.2 ng.-atoms per gram (= 0.095 p.p.m.) a value intermediate between the turkey and coturnix. We did not separate the eyes into component parts, as did Taussky et al. (1966), who reported that the iris contained the highest concentration, the retina and lens a large concentration, and the sclera, cornea, aqueous and vitreous humor the smallest concentration of selenium. Siren (1964) reported that the retinae of tern and roedeer contained 630 to 810 p.p.m. of selenium on a

dry weight basis (recalculated by us to be about 1,000 nanogram-atoms/g. fresh weight). He related these high concentrations of selenium to the very good visual acuity of these species. The significance of retinal selenium and its role as a photon receptor and energy transformer has been discussed by Aberg (1966). Based on low and variable results for selenium concentration in the human eye, Christian and Michaelis (1966) suggested that selenium probably plays no role in the visual process. Thus, the role of selenium in the visual process and neural physiology is ambiguous.

II. The distribution of ^{75}selenomethionine in chicken tissues.

Our last experiment measured the distribution of ^{75}selenomethionine in various organs of the male and female chicken. It was considered important to conduct this experiment, since the distribution of ^{75}selenium, as the selinite, appeared to concentrate in those organs possessing marked protein synthesis. The ^{75}selenomethionine analog was chosen because it had been demonstrated that it is incorporated into the various body proteins in the same manner as l-methionine.

Five male and five female chickens received approximately 150 microcuries of ^{75}selenomethionine intravenously via the brachial vein. One hour was allowed for uptake before killing the birds and removing the organs for analysis.

Results

Tables 5 and 6 present the data obtained from the males and females, respectively. The ^{75}selenomethionine was concentrated in those organs with marked protein synthetic properties. The distribution of this methionine analog was similar, but not identical, to that of ^{75}selenium. In both sexes the pancreas had the greatest concentration followed by the testes or oviduct, the kidney and the pituitary. The concentration of ^{75}selenomethionine within the pineal was about the same order of magnitude as for the liver, spleen and adrenals. The distribution results did not correlate with the total concentration of selenium for the organs. This is explainable on the basis that protein synthesis can occur at one rate, whereas the storage and release of the protein formed can be at an entirely different rate. It would appear that the pineal has a high selenium content because it incorporates sulfur and selenium containing amino acids into its protein, but that its protein turnover rate is rather slow allowing the high concentrations of selenium observed. It is quite possible that the selenium is located in the protein portion of the endoplasmic reticulum and not in the protein which is excreted, however in the case of the pancreas and testes or oviduct a portion of the protein containing selenium undoubtedly is eliminated.

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Table 1a. Endogenous selenium content in year old Leghorn males' tissue[1] (Experiment 1).

	Cerebellum	Liver	Blood	Kidney	Pineal
Fresh tissue	3.5 (19)	6.3 (19)	8.0 (18)	8.6 (19)	51.0 (19)
Distribution ratio organ Se/blood Se	.44 (18)	.79 (18)		1.08 (18)	6.38 (18)

[1] Mean values are expressed as ng.-atoms Se/g. of fresh tissue and as the ratio of organ to blood Se. The number of samples is enclosed in parentheses. Mean values connected by a continuous underline are not statistically significant at probability level of 0.05.

Table 1b. Tracer ^{75}Selenium localization in year old Leghorn males' tissue[1] (Experiment 1).

	Cerebellum	Blood	Pineal	Liver	Kidney
Fresh tissue	.05 (19)	.57 (19)	1.14 (19)	3.4 (19)	4.3 (19)
Organ ^{75}Se/Blood ^{75}Se	.09 (19)		2.0 (19)	6.0 (19)	7.6 (19)

[1] Mean values are expressed in counts 10^{-6}/min/g of fresh tissue and as the ratio of organ to blood ^{75}Se. The number of samples is enclosed in parentheses. Mean values connected by a continuous underline are not statistically significant at probability level of 0.05.

Table 2a. Endogenous Selenium content in 24 week old Leghorn males' tissue[1] (Experiment 2).

	Cerebellum	Blood	Liver	Kidney	Pineal
Fresh tissue	3.51 (27)	4.16 (28)	9.30 (27)	11.8 (28)	238. (26)
Distribution ratio organ Se/Blood Se	.84 (27)		2.24 (27)	2.84 (28)	57.2 (26)

[1] Mean values are expressed as ng.-atoms Se/g. of fresh tissue and as the ratio of organ to blood Se. The number of samples is enclosed in parentheses. Mean values connected by a continuous underline are not statistically significant at probability level of 0.05.

Table 2b. Tracer ^{75}Selenium localization in 24 week old Leghorn males' tissue[1] (Experiment 2).

	Cerebellum	Blood	Pineal	Kidney	Liver
Fresh tissue	.02 (28)	.17 (28)	.42 (26)	1.08 (28)	1.71 (28)
Distribution ratio organ ^{75}Se/Blood ^{75}Se	.12 (28)		2.47 (26)	6.36 (28)	10.09 (28)

[1] Mean values are expressed in counts 10^{-6}/min/g. of fresh tissue and as the ratio of organ to blood ^{75}Se. The number of samples is enclosed in parentheses. Mean values connected by a continuous underline are not statistically significant at probability level of 0.05.

Table 3. Comparison of endogenous selenium concentrations found in adult female chicken, turkey and coturnix parts (Experiment 3).

	Nanogram-atoms Se/gram weight					
Part	No.	Chicken	No.	Turkey	No.	Coturnix
Blood	(5)	2.3 ± 1.0	(4)	2.1 ± 0.3	(10)	5.8 ± 0.4
Pineal	(4)*	16.0	(4)*	4.6	(28)*	4.8
Pituitary	(5)*	14.7	(5)*	12.4	(12)*	14.0
Kidney	(5)	8.2 ± 1.3	(5)	13.6 ± 1.7	(10)	18.9 ± 0.6
Spleen	(5)	6.8 ± 0.5	(5)	8.9 ± 0.1	(10)	12.0 ± 0.6
Liver	(5)	6.0 ± 0.8	(5)	10.1 ± 0.1	(10)	11.2 ± 0.9
Pancreas	(5)	4.6 ± 0.6	(5)	6.3 ± 0.5	(10)	15.9 ± 0.9
Magnum	(5)	3.6 ± 0.5	(5)	2.3 ± 0.1	(8)	4.4 ± 0.6
Cerebrum	(5)	2.9 ± 0.6	(5)	2.9 ± 0.9	(10)	4.5 ± 0.7
Diencephalon	(5)	2.8 ± 0.4	(5)	2.4 ± 0.1	(9)	3.9 ± 0.2
Cerebellum	(5)	2.7 ± 0.3	(5)	3.8 ± 0.8	(10)	4.7 ± 0.6
Ovary	(5)	2.3 ± 0.5	(5)	4.6 ± 0.1	(10)	6.2 ± 0.4
Pectoral	(5)	2.2 ± 0.2	(5)	2.4 ± 0.6	(10)	3.8 ± 0.2
Feces	(5)	1.0 ± 0.5	(5)	1.6 ± 0.1	(10)	2.5 ± 0.3
Crop Contents	(5)	0.9 ± 0.3	(4)	1.6 ± 0.1	----	---- ----
Egg yolk	(2)	6.7			(5)	7.9 ± 1.9
Egg albumen	(2)	1.3			(5)	1.7 ± 0.4
Egg shell	(2)	0.85			(4)	1.8 ± 0.3
Ration		3.42		4.25		4.25

*Number of individual samples of pineal and pituitary pooled to provide sufficient material for analysis.

Table 4. Selenium concentrations of pineal, pituitary and eye of turkey and coturnix[1] (Experiment 4).

Fresh tissue	Turkey ♂		Coturnix ♀	
	No.	ng.-atoms Se/g.	No.	ng.-atoms Se/g.
Pineal	(23)	6.26	(48)	6.51
Pituitary	(36)	10.09	(25)	13.85
Eye, right	(19)	0.91 ± .07	(20)	1.51 ± .18

[1] Analyses of pineal and pituitary are means based on two or more pooled samples. The eyes from individuals in Experiment 3 are combined with those of Experiment 4 and summarized together.

Table 5. The Concentration of ^{75}selenomethionine and Total Selenium in the Organs of Five Male Chickens.

Organ	^{75}selenomethionine (CPM X 10^6)	Total selenium (ng Se/g)
Pancreas	2.422 ± .357	7.46 ± .99
Testes	1.910 ± .190	3.94 ± .40
Kidney	1.212 ± .066	13.30 ±1.24
Pituitary	1.111 ± .059	47.95 (5 pooled)
Liver	1.071 ± .044	9.25 ± .33
Adrenals	.924 ± .089	12.95 ±1.46
Pineal	.863 ± .125	23.98 (5 pooled)
Spleen	.741 ± .053	9.20 ± .77
Blood	.292 ± .014	5.54 ± .79
Cerebellum	.210 ± .017	3.57 ± .24
Diencephalon	.167 ± .009	4.88 ± .31
Cerebrum	.152 ± .013	4.12 ± .38
Retina	.141 ± .015	4.94 ±1.24
Pectoral muscle	.078 ± .008	3.12 ± .18

Table 6. The Concentration of ^{75}selenomethionine and Total Selenium in the Organs of Five Female Chickens.

Organ	^{75}selenomethiopine (CPM X 10^6)	Total selenium (ng Se/g)
Pancreas	2.980 ± .330	10.81 ± .71
Oviduct)magnum)	1.380 ± .360	9.15 ±1.29
Kidney	1.380 ± .120	12.32 ±1.55
Pituitary	1.360 ± .100	29.00 (5 pooled)
Pineal	1.250 ± .120	30.29 (4 pooled)
Liver	1.041 ± .090	10.29 ± .35
Spleen	.693 ± .106	11.15 ± .93
Adrenals	.769 ± .059	20.25 ±5.54
Blood	.445 ± .034	3.94 ± .36
Ovary	.352 ± .018	6.27 ± .67
Cerebellum	.193 ± .010	7.65 ± .40
Diencephalon	.176 ± .004	11.25 ±1.78
Cerebrum	.156 ± .004	6.10 ± .97
Retina	.153 ± .011	8.49 ±1.70
Pectoral muscle	.117 ± .014	4.40 ± .33