a previous publication(9) that raw navy beans were poorly digested *in vitro* by trypsin as compared to autoclaved beans.

Haines and Lyman(10) proposed that the soybean trypsin inhibitor acts by stimulating the pancreas to secrete excessive amounts of enzymes, thus creating partial deficiencies of the most limiting amino acids in the animals fed soybean meal. It may be that the navy bean trypsin inhibitor as a factor present in raw navy beans also stimulates the production of pancreatic enzymes resulting in the loss of critical amino acids through feces. Another possibility of explaining the unavailability of critical amino acids is that there may be present a factor in raw navy beans which inhibits the production by the pancreas of the necessary digestive enzymes, thus making the intestinal proteolysis incomplete, as postulated by Saxena *et al*(3) in explaining the low nutritive value of raw soybeans. However, morphological changes observed in the pancreas of animals fed raw navy beans did not allow differentiation between these two mechanisms, because the condition in which the cytoplasm of the pancreas was devoid of secretory granules could arise from either mechanism of action described.

In the light of the above discussion, it can be concluded that the growth inhibition of rats fed raw navy beans could be partly due to the impairment in the availability of critical amino acids and partly due to the methionine deficiency. Moreover, this condition can be further aggravated by the low food intake resulting in the limited supply of amino acids in the animals fed raw navy beans.

Summary. An experimental diet of raw navy beans produced changes in rats which included: growth inhibition, reduced food consumption, pancreatic acinar atrophy, fatty metamorphosis of the liver, thyroid follicular atrophy, and hyperkeratosis of the esophagus and skin. Significant morphological changes were not observed in the organs of rats fed an autoclaved navy bean diet.

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Changes in Metal Distribution of the Avian Oviduct During the Ovulation Cycle.* (30341)

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The elucidation of the mechanisms of metal transport in biological systems has been of long and continued interest(1). Many systems have been studied for this purpose and among them the avian shell gland appears to be especially suited since in the domestic fowl this tissue produces in 20 hours a shell which contains about 2.0 g of calcium(2). Investigation of this highly amplified system of calcium transport may provide information which would be of general application to transport theory. In the present investiga-

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tion, the shell gland, the isthmus, and the magnum segments of the domestic fowl's oviduct have been utilized in order to detect differences in the concentration of this and other metals in the segments that may occur at various phases of the ovarian cycle. The study was undertaken prior to an attempt to define some of the cellular constituents and control mechanisms that play a role in the movement of calcium.

Methods. Twenty-five single comb White Leghorn hens were sacrificed and exsanguinated by decapitation. Oviducts were removed, cut into sections corresponding to magnum, isthmus, and shell gland. The birds were so selected that specimens were obtained from 3 sets of 5 animals each, where each set was composed of animals with an egg located in one of the above-mentioned segments at the time of sacrifice. Another set of 5 animals represented immature chickens which had not yet laid an egg. All oviduct segments from the experimental animals were weighed wet, quickly frozen, vacuum dried immediately from the frozen state, and weighed in the dry state. The dry segments were then ashed at 500°C for 5 hours.

Metal analyses were performed by emission spectroscopy. For standards, an appropriate mixture of MgO, $CaCO_3$, Fe_2O_3, CuO, ZnO, and MnO was successively diluted in steps of 1:1 with a matrix of 70% $KHPO_4$ and 30% NaCl. Each standard was mixed with an internal standard and $LiBO_4$ containing 4% SiO_2 in the ratio of 5:1:10 and fused at 850°C for 10 minutes in graphite crucibles. The internal standard was 7% Co_3O_4 in $LiBO_2$. After fusion, the resulting bead was crushed and mixed with graphite in the ratio of 1:1, packed into ⅛″ preformed electrodes and burned in a DC arc. Samples were treated in the same manner. Arcing conditions, photographic technique, line pairs, etc., are the same as were reported by Joensuu and Suhr(3).

Analytical data were collected for per cent ash, and for quantity of the following metal constituents on an ash basis: calcium, zinc, iron, magnesium, manganese, and copper. To corroborate the extensive literature on sodium and potassium as major tissue cations, the two elements were determined by flame photometry(4) in 25 samples. Results for all analyses represent a summation of intra- and extracellular cations.

Results and discussion. Ash content. Data collected from determination of tissue ash content are expressed as mg/g on a dry weight basis and are shown in Table I. The mean value and standard error for all magnum tissue is 35.2 ± 1.8 mg/g; for all isthmus tissues, 43.4 ± 3.1 mg/g; and for all shell gland samples, 65.1 ± 1.5 mg/g. The latter figure is significantly the highest. Thus it appears from the data that the highest concentration of non-volatile mineral constituents, judged on a dry weight basis, is present in the shell gland.

In an effort to detect fluxes of non-volatile metals, parallel functional segments were compared from hens with eggs located in various segments of the oviduct (Table I). The only significant difference that appeared in the data of the normal adult samples occurred in the isthmus. Wide variations in isthmus ash content were noted when an egg was located in the infundibulum (45.8 ± 7.6 mg/g) and the magnum (40.0 ± 6.3 mg/g), but a high narrow range of values was recorded (52.5 ± 1.4 mg/g) at the time the egg was located in the isthmus.

It is interesting that the ash content of all immature chicken tissues shown in Table I is high in all sections when compared to mean values of adult phases. When such values for sections are compared, the shell gland content (77.9 ± 6.8 mg/g) was highest with lower isthmus (55.8 ± 1.7 mg/g) and magnum values (51.3 ± 6.8 mg/g).

Major cations. Analyses of 25 ashed tissue samples were made by flame photometry for sodium and potassium, the expected major cations. Based on per cent ash weight the following mean values resulted: magnum, 9.9% sodium and 22.1% potassium; isthmus, 11.1% sodium and 22.2% potassium; shell gland, 13% sodium and 22.5% potassium. A detailed study of sodium and potassium was not the intent of this experiment.

Calcium content. Data for calcium content of tissue are shown in Table I. The mean calcium content for all magnum tissues is

12.7 ± 0.7 mg/g; for isthmus tissues, 59.6 ± 7.0 mg/g; and for shell gland, 23.6 ± 2.1 mg/g. High isthmus calcium values have been reported by Taylor and Hertelendy(5). A previous histochemical study(6) indicated evidence of negligible amounts of calcium in isthmus and shell gland with slightly greater quantities in the shell gland. However, the unique finding of this study is the high isthmus concentration of calcium found when the egg is located in the infundibulum and the magnum with values of 61.5 ± 6.2 mg/g and 77.3 ± 15.1 mg/g ash, respectively. This relationship was discovered by comparing isthmus sample analyses during different phases of the laying cycle. Also interesting

TABLE I. Ash and Metal Content of Oviduct Segments in Relation to Egg Location at Time of Sacrifice.

Oviduct segment	Location of egg at sacrifice	Ash†	Fe‡	Ca‡	Mg‡	Cu‡	Zn‡	Mn‡
		mg/g				μg/g		
Magnum	Immature*	51.3 ± 6.8	7.8 ± .5	10.0 ± 1.8	20.5 ± 4.4	82 ± 13	796 ± 49	40 ± 9
Isthmus	" *	55.8 ± 1.7	8.3 ± 1.0	30.8 ± 4.1	13.2 ± 1.0	216 ± 23	1054 ±339	51 ± 5
Shell gland	" *	77.9 ± 6.8	13.2 ± 2.0	20.8 ± 5.6	14.6 ± 1.3	95 ± 11	949 ± 36	62 ± 10
Magnum	Infundibulum	31.4 ± 1.0	.7 ± .2	15.3 ± .9	52.3 ± .5	115 ± 17	1133 ± 88	77 ± 5
"	Magnum	35.4 ± 2.8	2.6 ± 1.7	12.2 ± 1.3	42.5 ± 1.3	189 ± 65	902 ± 82	68 ± 5
"	Isthmus	Samples lost						
"	Shell gland	37.8 ± 3.6	.9 ± .1	11.2 ± 1.2	40.2 ± 5.6	101 ± 3	942 ± 42	71 ± 4
"		35.2 ± 1.8§	1.5 ± .06§	12.7 ± .7§	44.5 ± 2.5§	137 ± 25§	980 ± 47§	71 ± 3§
Isthmus	Infundibulum	45.8 ± 7.6	2.3 ± 1.5	61.5 ± 6.2	19.0 ± .9	443 ± 47	900 ± 88	57 ± 6
"	Magnum	40.0 ± 6.3	3.7 ± 1.3	77.3 ± 15.1	17.5 ± 1.0	372 ± 44	780 ± 87	60 ± 10
"	Isthmus	52.5 ± 1.4	7.4 ± .6	38.5 ± 5.5	14.0 ± .3	255 ± 45	705 ± 55	80 ± 30
"	Shell gland	41.7 ± 2.8	.9 ± .1	41.8 ± 5.1	18.2 ± 1.5	358 ± 23	1025 ± 29	58 ± 4
"		43.4 ± 3.1§	3.1 ± .8§	59.6 ± 7.0§	17.6 ± .7§	348 ± 33§	862 ± 49§	61 ± 5§
Shell gland	Infundibulum	63.3 ± 6.5	3.7 ± 2.5	28.5 ± 4.0	22.0 ± 1.4	83 ± 10	1050 ± 65	93 ± 11
"	Magnum	68.8 ± .9	5.8 ± 2.0	26.8 ± 3.6	19.8 ± 2.0	92 ± 9	860 ±105	105 ± 14
"	Isthmus	65.5 ± .8	11.1 ± 1.9	19.0 ± .9	16.5 ± .7	110 ± 10	980 ± 20	87 ± 13
"	Shell gland	61.1 ± 4.8	1.6 ± .1	16.0 ± 1.2	18.2 ± 1.4	113 ± 13	1150 ± 43	74 ± 5
"	"	65.1 ± 1.5§	4.8 ± 1.2§	23.6 ± 2.1§	19.6 ± 1.0§	97 ± 8§	995 ± 54§	92 ± 7§

Values presented as means ± standard errors.

* No egg.
† Dry wt basis.
‡ Ash wt basis.
§ Grand mean ± standard error of segment.

is the large standard deviation for the mean value of the isthmus calcium content when the egg is in the magnum. This may be indicative of a temporary accumulation of metal in the tissue during the phase when the egg is approaching the isthmus segment. The large standard deviation may also be due to a pooling effect since no attempt was made to discriminate as to whether the egg was high or low in the magnum segment. Since values fall significantly for isthmus tissue when the egg is located in the isthmus (38.5 $\pm$ 5.5 mg/g) or in the shell gland (41.8 $\pm$ 5.1 mg/g), it is tempting to speculate that tissue storage of calcium and subsequent movement of the metal do occur in isthmus tissue. A high concentration of citric acid has also been found in the isthmus and it has been suggested that the high calcium and citrate content may be involved with the primary calcification of the mammilae(7).

Comparison of tissue samples in any one segment (Table I) indicates the possibility of delivery of calcium in the isthmus, since values for isthmus tissue are high when the egg is in the infundibulum (61.5 $\pm$ 6.2 mg/g), and significantly lower when the egg is in the isthmus (38.5 $\pm$ 5.5 mg/g). Further support of calcium transport by the isthmus has been shown by other experiments in which intramuscular injections of Ca^{45} produced significant radioactivity on the shell membrane of an isthmus egg 10 minutes after injection(9). Oshima and Nozaki(8) in a study of albumin secretion in the magnum report the transport of some calcium to the egg in this segment. Although the values are not as high for shell gland specimens, similar evidence of calcium delivery to or with albumin in the magnum was noted.

Calcium concentration of immature tissues is generally lower when compared to adult specimens.

The data collected from calcium analyses indicate that the isthmus may play an important role in the movement of this metal. The mechanism and role of the cellular constituents of the tissue cannot be defined, however, from this type of data.

Magnesium content. Since magnesium is a divalent metal intimately connected with protein synthesis, and is associated with intracellular events(10), the concentrations of the metal in the various oviduct segments are also of interest, especially in relation to fluxes that might be observed by comparing any of the 3 functional segments, or by intracomparing samples of any one segment with respect to egg position at the time of sampling. The results shown in Table I indicate that magnesium concentrations are highest in magnum tissues (44.4 $\pm$ 2.5 mg/g ash) and that low levels are found in the isthmus (17.6 $\pm$ 0.7 mg/g ash) and shell gland (19.6 $\pm$ 1.0 mg/g ash) segments; such a concentration pattern is similar to that reported by Taylor and Hertelendy(5). The data further indicate that magnesium concentration fluctuates in magnum tissue depending upon egg position. This fluctuation is not noted in the isthmus and shell gland where magnesium levels are fairly constant and apparently independent of processes connected with the passage of the egg. Magnum magnesium values are highest when the egg is in the infundibulum and show a tendency to fall in concentration as the egg moves down the oviduct. The high magnesium values in the magnum may be related to the high levels of protein synthesis in that segment. In addition, the tendency for magnesium concentrations in the magnum to fall with the passing of the egg through the segment may indicate that magnesium may be carried with the secreted albumin to the egg in the magnum. In this connection, it is interesting to note the low magnesium concentration in the immature magnum, which has not begun albumin secretion. It is known that the protein synthesized in the magnum is secreted as egg albumin to the egg structure(11).

Iron content. Iron analyses were undertaken as an index of the contribution by residual blood in the vascular system of the segments to the metal analyses. Although animals were rapidly exsanguinated at sacrifice by decapitation, there was a necessity for establishing some measure of the contribution of the blood to the total calcium content of the segments analyzed. For such purposes, the pattern of iron content of the tissues observed was compared with the pat-

terns of other metals. It was judged that a similar fluctuation of the patterns of iron and calcium would indicate that the blood was making a substantial contribution to the calcium, or other metal, being observed. As was the case, the absence of coincident fluctuation of calcium and the other elements with iron was interpreted to mean that very little contribution to calcium tissue content was made by residual blood.

The high iron concentrations in isthmus and shell gland tissue (7.4 ± 0.6 and 11.1 ± 1.9 mg/g ash, respectively, in Table I) when the egg was in the isthmus are difficult to explain at present. Similarly, the high concentrations of iron in all immature segments cannot be explained without further investigation of iron distribution in subcellular or extracellular compartments.

Copper content. The mean copper content (Table I) for all magnum tissues is 137.1 ± 25.3 μg/g; for isthmus tissue, 348.1 ± 32.9 μg/g; and for shell gland, 97.2 ± 7.8 μg/g. The report of the distribution of copper is unique to this study and the fact that isthmus tissue values are significantly higher than either magnum or shell gland tissue is of considerable interest.

When isthmus samples are compared, it is interesting to note that values fall significantly during the passage of the egg from the infundibulum through the isthmus and rise when the egg is in the shell gland. This unusual finding merits further study. The fall in copper during the egg passage may indicate a transfer of copper to the egg. The lowest isthmus value is still significantly higher than was found in either of the other segments. The generally high copper levels in the isthmus tissue, which occur irrespective of egg location, may indicate a high cuproenzyme and/or cuproprotein concentration(12).

Zinc content. Initially, zinc was of particular interest since the enzyme carbonic anhydrase is zinc-containing and is believed to play a role in the precipitation of calcium carbonate of the shell(13). Noteworthy is the fact that zinc was found in all functional segments and not just in the shell gland or the isthmus. Its ubiquitous appearance at similar concentration levels in all of the tissues complicates interpretation of its functional role.

Manganese content. The trace element, manganese, was present in all segments. A fall in magnum manganese values was noted as the egg passed from the infundibulum to the shell gland suggesting transport of the metal to the egg in the magnum. No significant differences were observed in isthmus analyses. Shell gland values were highest, as is illustrated by comparing grand mean values of manganese for all segments. A significant drop in manganese values of shell gland tissue was noted from the time the egg was in the magnum until after it entered the shell gland. The data suggest that manganese is added to the shell structure in the shell gland.

Summary. A study was made of the metal distribution in certain functional segments (magnum, isthmus, and shell gland) of the oviduct of the domestic fowl (*Gallus domesticus*) in an effort to detect patterns of metal flux in these tissues which might be associated with calcium transport. Concentrations of calcium, magnesium, iron, manganese, copper, and zinc were determined by spectral emission analysis of tissue ash from birds sacrificed at various phases of the ovulation cycle. Ash content was highest in the shell gland; calcium and copper concentrations were elevated in the isthmus and magnesium was elevated in the magnum. There appears to be a relationship between the metal concentrations and the known metabolic functions of the oviduct segment analyzed. The data also indicate that 1) the movement of trace amounts of the metals, zinc, manganese and magnesium, to the egg occurs in particular segments; 2) high copper levels are present in the isthmus; 3) the isthmus transports calcium for deposition on the shell membrane; 4) potassium and sodium are major cations of all segments analyzed.

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Effect of Methionine on Nicotinic Acid and Indoleacetic Acid Pathways of Tryptophan Metabolism *in vivo*. (30342)

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Previous clinical studies from this laboratory have revealed that an oral test load-dose of DL-methionine following oral intake of a monoamineoxidase inhibitor (tranylcypromine) markedly increased certain urinary indoles above those levels obtained with the monoamineoxidase inhibitor alone. The indoles in question were found to be tryptamine and the glucuronide conjugate of indoleacetic acid as measured by indoleacetamide(1). Tryptamine is known to be an intermediary metabolite of tryptophan metabolism capable of being oxidized to indoleacetic acid by monoamineoxidase in animal tissues as well as in intestinal bacteria(2). To investigate further the methionine effect *per se* on tryptophan metabolism, rats were studied under controlled dietary conditions using synthetic diets. It is the purpose of this paper to report the effect of excess methionine on tryptophan metabolism *in vivo* as measured by urinary N^1-methylnicotinamide (MNA) and indoleacetic acid (IAA), 2 metabolites originating from tryptophan, by different metabolic pathways(3). Measurement of urinary creatinine (CR) was also included in this study because of the involvement of methionine in its formation.

Materials and methods. Forty-eight Sprague-Dawley male rats, approximately 55 days old, weighing about 200 g, and maintained for 7 to 10 days prior to experimentation on a control 30% casein diet (CAS),* were divided into 6 groups of 8 rats per group. Each group was placed on an appropriate test diet. In some instances more than one group of 8 rats was run with a given diet. The test diets used were as follows: (1) diet (CAS)* = 30% casein control; (2) diet (CS) = complete-synthetic amino acid diet

* Composition of 30% casein diet (CAS) in g/100 g of diet was: casein, 30.0; corn starch, 46.0; yellow dextrin, 2.0; non-nutritive fiber (cellulose), 4.0; NRRL salt mix No. 446(4), 3.0; cottonseed oil, 14.0, and vitamin supplement in corn starch, 1.0. One gram of corn starch-vitamin supplement supplied the following vitamins in mg/100 g of diet: p-aminobenzoic acid, 11.02; ascorbic acid (coated 97.5% ascorbic acid), 101.75; biotin, 0.04; Ca pantothenate, 6.61; choline dihydrogen citrate, 371.52; folic acid, 0.20; i-inositol, 11.02; menadione, 4.96; nicotinic acid, 9.92; pyridoxine • HCl, 2.20; riboflavin, 2.20; thiamin • HCl, 2.20; vit. A (dry stabilized, 500,000 U.S.P. units/g), 3.97; vit B_{12} (0.1% in mannitol), 2.98; vit D_2 (dry, 500,000 U.S.P. units/g), 0.44; vit E acetate (25%, 250 I.U. vit E/g), 48.50; and corn starch q.s., 420.47. This diet was prepared by General Biochemicals.