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Storage, Retention and Distribution of Folic Acid in the Chick.*

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The water-soluble vitamins are generally stored and retained by animal tissues to a much smaller extent than the fat-soluble vitamins. High oral intakes of vitamin A are known to produce high concentrations of this vitamin in the livers of rats and once established these stores are available to the animal for long periods of time following the exclusion of vitamin A from the diet.[1] Data on the storage and retention of the B vitamins are limited. Schultz *et al.*[2] were unable to find a further increase in the body concentration of thiamine after a dietary level of 65 γ of thiamine per rat per day was reached. Pearson *et al.*[3] found that an increased dietary level of pantothenic acid had no significant effect on the level of this vitamin in the livers of chicks but did produce increases in the leg and breast muscle.

In the course of our studies on folic acid in chick nutrition we were interested in the storage, retention, and distribution of this vitamin in the chick. Results of these investigations are reported here.

Experimental. Day-old White Leghorn cockerels obtained from a commercial hatchery were maintained in electrically-heated cages with raised screen bottoms. All chicks were fed our folic acid-deficient basal ration 494-K which consists of dextrin 61 g, alcohol-extracted casein 18 g, gelatin 10 g, soybean oil 5 g, salts V 6 g, cystine 0.3 g, thiamine 0.3 mg, riboflavin 0.6 mg, nicotinic acid 5.0 mg, pyridoxine hydrochloride 0.4 mg, calcium pantothenate 2.0 mg, inositol 100 mg, choline chloride 150 mg, biotin 0.02 mg, 2-methyl-1,4-napthoquinone 0.05 mg, and α-tocopherol 0.3 mg. Each chick received in addition one drop per week of fortified haliver oil containing 120 A.O.A.C. units of vitamin D_3 and 1200 U.S.P. units of vitamin A per drop. Synthetic folic acid (pteroyl glutamic acid) was used in all experiments. Food and water were given *ad libitum.* The chicks were maintained on the above ration for 3 to 4 days, weighed, and chicks within a 10 g weight range were distributed uniformly into the experimental groups.

In the first series groups of 8 chicks each were placed on folic acid levels of 0, 25, 100, and 1000 γ per 100 g of ration. The number of chicks per group was reduced to 6 after the first week by discarding the heaviest and lightest animal from each group. The animals were sacrificed when 4 weeks old and representative samples of liver and muscle tissue were taken from 4 chicks in each group for folic acid analyses. Average weights of the animals at 4 weeks, number of survivors, and folic acid values for the liver and muscle tissues are summarized in Table I.

In a second series, 4 groups of 16 chicks each were placed on the following diets: unsupplemented basal, basal plus 200 γ of folic acid per 100 g of ration, basal plus 1000 γ of folic acid per chick per day for 5 days (total of 5 mg per chick) by intraperitoneal injection, and basal plus 1000 γ of folic acid per chick per day for 5 days orally. Four chicks from each group were taken for folic acid analysis of the liver and muscle tissues at the end of 1, 3, and 5 weeks from the time the supplements were started. In addition, folic acid analyses were made on representative tissues from 3 series of 4- to 5-week-old

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[2] Schultz, A. S., Light, R. F., Caacas, L. J., and Atkin, L., *J. Nutrition*, 1939, **17**, 143.

[3] Pearson, P. B., Melass, V. H., and Sherwood, R. M., *J. Nutrition*, 1946, **32**, 187.

TABLE I.
Folic Acid Storage in Chick Liver and Muscle with Increasing Dietary Intakes of Folic Acid.

Group No.	Supplement added per 100 g ration 494-K	Avg wt at 4 wk, g	γ of folic acid per g of Liver	Muscle
1	0	130 (6)*	1.07 (.79-1.52)†	.05 (.042-.069)
2	25 γ folic acid	175 (5)	2.35 (1.46-3.08)	.06 (.04-.07)
3	100 γ " "	190 (6)	3.52 (1.97-4.51)	.06 (.05-.08)
4	1000 γ " "	175 (6)	14.7 (11.9-16.4)	.04 (.03-.05)

* Numbers in parentheses refer to number of chicks surviving at 4 weeks.
† Numbers in parentheses give the range of values found.

chicks receiving 200 γ of folic acid per 100 g of our basal ration. The results of these series are summarized in Tables II and III.

Samples taken for analysis were minced in a Potter-Elvehjem homogenizer[4] or a Waring Blendor. Liver and muscle samples from chicks in series I and II were digested with taka-diastase in pH 4.5 acetate buffer for 24 hours at 37°C since this procedure has been found satisfactory for liberating bound folic acid in these tissues.[5,6] The other body tissues were likewise treated with taka-diastase and in addition duplicate series were treated in pH 4.5 acetate buffer with a kidney conjugase preparation prepared according to the procedure of Bird *et al.*[7] No significant differences in liberation of folic acid from the various tissues was noted with either procedure and the values given (Table III) represent an average of values from tissues receiving the 2 liberation procedures. Folic acid was determined with *Streptococcus faecalis* using the medium of Luckey *et al.*[8] modified by the addition of 1.0 cc of Speakman's[9] salts B per 100 cc of medium.

Results and Discussion. The results of the first series demonstrate that increasing the dietary level of folic acid has little effect on the content of this vitamin in chick muscle tissue since muscle folic acid values remained relatively constant regardless of dietary intake. The folic acid content of the liver, however, increased directly with increased dietary intakes of the vitamin. Liver values were 1.07, 2.35, 3.52 and 14.7 γ of folic acid per g of fresh liver for the 0, 25, 100 and 1000 γ dietary level groups respectively. The values presented here are not intended to represent absolute amounts of folic acid that will be found in chick liver and muscle tissue on similar folic acid intakes on different diets; but they merely represent the relative efficiency of storage of folic acid under the particular standardized conditions used in this series (Series I). The observed increase of folic acid in chick liver as compared to the constancy of chick muscle tissue follows the expected storage of a B vitamin and is in contrast to the increased storage of pantothenic acid in muscle tissue reported by Pearson *et al.*[3]

Results from the second series indicate that high initial dosages of the vitamin are stored inefficiently in the liver since only 3.6 γ of folic acid per g of liver were present in chicks from Groups 3 and 4 (Table II) 2 days after administration of the final mg of folic acid. Muscle folic acid values remained fairly constant in Groups 1, 2 and 4 but a temporary increase occurred in the injected group. The control group of chicks

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5 Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *Univ. Texas Publ.*, 1942, No. 4237, 32.

6 Luckey, T. D., Briggs, G. M., Jr., Moore, P. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, **161**, 395.

7 Bird, O. D., Robbins, M., Vanderbelt, J. M., and Pfiffner, J. J., *J. Biol. Chem.*, 1946, **163**, 649.

8 Luckey, T. D., Briggs, G. M., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **152**, 157.

9 Speakman, H. B., *J. Biol. Chem.*, 1923-24, **58**, 395.

TABLE II.
Folic Acid Content of Liver and Muscle Tissue from Chicks Receiving Various Folic Acid Supplements.

Method and amount of supplementation	Avg weight and folic acid values in γ/g								
	1st week*			3rd week			5th week		
	Avg wt, g	Folic acid in Liver	Folic acid in Muscle	Avg wt, g	Folic acid in Liver	Folic acid in Muscle	Avg wt,‡ g	Folic acid in Liver	Folic acid in Muscle
0	85	.80 (0.4-1.2)†	.05 (.04-.06)	95	1.1 (0.7-1.1)	.04 (.04-.05)	85	1.0 (0.4-2.1)	.09 (.05-.13)
200 γ folic acid per 100 g basal diet	90	2.1 (1.2-2.7)	.05 (.04-.06)	215	1.6 (1.2-1.9)	.06 (.05-.08)	410	3.9 (3.6-4.3)	.07 (.07)
1 mg folic acid per day for 5 days by injection	90	3.6 (2.8-5.0)	.27 (.21-.34)	220	1.2 (1.2-1.7)	.09 (.07-.12)	395	0.6 (.45-.70)	.10 (.09-.16)
1 mg folic acid per day for 5 days orally	90	3.6 (1.4-5.0)	.08 (.06-.09)	210	0.7 (0.5-0.7)	.06 (.05-.07)	385	0.34 (.30-.40)	.12 (.09-.18)

* Weekly periods are given from the time supplementation was started.
† Numbers in parentheses refer to range of folic acid values.
‡ Number of deaths during the 5 week period was 1, 0, 0, and 0 for groups 1, 2, 3, and 4 respectively.

TABLE III.
Folic Acid Content of Tissues from 4-5-Week-Old Chicks.

Tissue	Folic acid in γ/g*
Liver	3.05 (1.8-4.3)†
Kidney	1.6 (1.0-2.2)
Pancreas	.95 (.4-1.5)
Testes	.6
Spleen	.28 (.19-.27)
Heart	.21 (.19-.23)
Brain	.19 (.17-.2)
Lung	.17 (.10-.24)
Muscle	.08 (.04-.12)
Skin	.05 (.05-.06)

* Each value represents the average of 6 samples, each of which consisted of the pooled tissue of 2 chicks (total of 12 chicks).
† Numbers in parentheses refer to range of folic acid values encountered.

which received the 200 γ level of folic acid had liver and muscle folic acid values in fair agreement with those obtained in the first series. The failure of the chicks on the 200 γ dietary level of folic acid (Table II) to show the expected increases in liver folic acid values over the groups receiving the 100 γ dietary level (Table I) suggests more efficient storage of folic acid at an early age in the slower growing animals of the first series. No significant decreases in liver folic acid occurred in the basal group and the 0.6 and .36 γ/g drop from the 3rd to the 5th week in Groups 3 and 4 respectively is small compared to the 2.4 and 2.9 γ/g drop that occurred from the 1st to the 3rd week. Further, the increased growth from the 3rd to the 5th week as compared to the 1st to the 3rd week would act to distribute the vitamin over a larger weight of tissue and the decreases in the total amount of liver folic acid would actually be smaller than is indicated by the values given. The folic acid in the basal ration (3 to 4 γ per 100 g) plus that produced from a limited amount of intestinal synthesis would aid slightly in maintaining liver stores. Nevertheless, the ability of the injected and orally administered groups to continue growing in spite of the fact that their liver and muscle tissues were as low in folic acid as the corresponding tissues of the basal group is interesting. Explanations for this discrepancy are lacking, but one possibility is that the initial high dosages of folic acid were used in the synthesis of a biologically active but microbiologically inactive compound. This view has been taken previously by Wright *et al.*[10] who observed a decrease of folic acid (as measured microbiologically) in incubated rat liver tissue. In any event, low tissue levels of folic acid are utilized efficiently on low folic acid diets. The possibility of storage in some other tissue of the animal seems improbable since analysis of the various body tissues of chicks receiving 200 γ of folic acid per 100 g of diet showed the liver to be the main site of storage.

Results from the second series also demonstrate that high initial doses of orally administered folic acid are used as efficiently as equivalent doses of injected folic acid. Group 4, which received the folic acid supplement by the oral route, gave a growth response at 3 weeks equivalent to that of the injected group (Group 3); and at 5 weeks the growth of the 2 groups plateaued at the same weight (difference of 10 g is not considered significant). These results do not substantiate the findings of Frost and Dann[11] who found differences in favor of the injected folic acid but a direct comparison of the data is impossible since different technics and animals were involved.

Liver, kidney, and pancreas were found to have the highest and skin and muscle the lowest content of folic acid; testes, spleen, heart, brain, and lung had intermediate values. The relative content of folic acid in chick liver, heart, and brain agree with the values found by Williams *et al.*[12]

Summary. High oral intakes of folic acid are stored inefficiently in chick livers and have little if any effect on the content of this vitamin in chick muscle tissue. Chicks given large oral doses of folic acid and then placed on a folic acid-deficient diet grew as well as chicks receiving an equivalent amount of folic acid by injection. The distribution of folic acid in some representative chick tissues has been studied.

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Intrasplenic Transplantation of Testes in Castrated Mice*

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Granulosa-cell tumors and luteomas have arisen in intrasplenic transplants of ovaries in castrated male and female mice.[1,2] The pathogenetic factors responsible for these ovarian tumors have been assumed to be (1) the capability of the liver to inactivate ovarian hormones, and (2) the increase in the amount of gonadotrophic hormones that

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