Summary. Visible precipitin bands have been demonstrated by double diffusion in agar studies with human urinary proteose and nephritic sera. These reactions were observed in autologous and cross systems. It appears that sera and urinary proteose obtained from patients with the nephrotic syndrome are the most likely to react.

1. Levine, J. M., Dubin, A., Armstrong, S. H., Jr., *J. Lab. and Clin. Med.*, 1959, v53, 167.

2. Korngold, L., *Ann. N. Y. Acad. Sci.*, 1957, v64, 681.

3. Oakley, C. L., Fulthorpe, A. J., *J. Path. and Bact.*, 1953, v65, 49.

4. Goodman, M., Greenspan, S. A., Krakower, C. A., *J. Immunol.*, 1955, v75, 96.

5. Baxter, J. H., Goodman, H. C., *J. Exp. Med.*, 1956, v104, 467.

6. Liu, C. T., McCrory, S., *J. Immunol.*, 1958, v81, 492.

7. Witebsky, E., Rose, N. E., Shulman, S., *Lancet*, 1958, v1, 808.

Received July 26, 1960. P.S.E.B.M., 1960, v105.

Effect of *Sterculia Foetida* Oil on Uptake of Water by the Avian Egg Yolk.*† (26110)

A. R. DOBERENZ, D. L. SCHNEIDER, A. A. KURNICK, M. G. VAVICH AND A. R. KEMMERER

Departments of Agricultural Biochemistry and Poultry Science, University of Arizona, Tucson

Addition of sterculic acid(1,2), *Sterculia foetida* oil(1), malvalic acid(2), or cottonseed oil(3) to the diet of laying hens, causes pink discoloration in cold storage eggs. Schaible and Bandemer(4) showed that the pink color was imparted by a complex of iron with conalbumin. Evans, *et al.*(5) using paper electrophoresis reported that protein migrated from the white to the yolk during storage. This suggests changes in the egg which allow passage of iron and conalbumin across the vitelline membrane(4). The purpose of this investigation was to determine the effect of *Sterculia foetida* oil on the water passage into intact yolks *in vitro*.

Experimental. A preliminary experiment was designed to study the effect of "clutch size" on water uptake by the yolk. In Experiment I a group of 65 randomly selected S. C. White Leghorn hens were housed in individual wire cages and fed a practical laying diet *ad libitum*. Eggs were collected daily at 3:00 P.M. for 21 consecutive days and stored in the refrigerator at 7°C overnight. The next morning the eggs were tested for water uptake by the method of Orru(6). The intact yolk was placed in a tared beaker and weighed. Deionized water was added and the contents incubated at 37.0 ± 1.0°C for 3 hours. Following incubation the liquid was removed from the beaker with the aid of an aspirator and blotted with filter paper. The beaker and its contents were weighed and the increase in weight was considered to be water uptake.

On the basis of the clutch size data (Table I), hens from Exp. I averaging 2, 3, and 4 day clutches were divided randomly into 4 lots of 8 or 9 birds each for Exp. II. Housing, laying diet, collection of eggs, and method for determination of water uptake by egg yolks were similar to procedures employed in Exp. I with the following exceptions: Gelatin capsules containing supplements of *Sterculia foetida* oil‡ or corn oil§ were administered daily. Hens from Lots 1 and 2 received a supplement of 0.30 g of corn oil daily, while Lots 3 and 4 received a mixture of 0.15 g *Sterculia foetida* oil and 0.15 g corn oil. The

* Supported in part by a grant from Mead Johnson & Co., Evansville, Ind.

† Preliminary report presented in *Fed. Proc.*, 1960, v19, 221.

‡ Extracted from the whole ground nut of the Java Olive tree, *Sterculia foetida*.

§ Mazola Oil, Corn Products Co., New York.

TABLE I. Influence of Clutch Size on Water Uptake of Egg Yolks and on Yolk Weights.

Size of clutch, eggs	No. of hens	Avg water uptake of yolks, g*	Avg yolk wt, g*
1	8	1.41 ± .28[a]† (50)‡	19.84 ± 1.54[a]† (53)‡
2	16	1.06 ± .26[b] (129)	18.68 ± 1.26[b] (144)
3	18	.99 ± .23[b] (190)	18.73 ± 1.02[b] (218)
4	8	.92 ± .15[b] (86)	18.44 ± .96[b] (96)

* Means with different superscripts are significantly different ($P < .01$).
† Mean ± stand. dev.
‡ No. of yolks tested.

eggs were stored at 7°C overnight (Lots 1 and 3) or for one month (Lots 2 and 4). In Lots 2 and 4 incubation time was reduced from 3 to 2 hours because many of the yolks ruptured during the 3 hour incubation period. The Halphen test(7) was used to determine presence of *Sterculia foetida* oil in egg yolks. Measurement of water uptake by intact yolks was begun when egg yolks from Lots 3 and 4 gave a positive Halphen test.

Results. The influence of average clutch size on water uptake of egg yolks from hens fed a practical laying diet (Exp. I) is shown in Table I. Water uptake data on yolks obtained from hens with an average of more than 4 eggs per clutch were not included because of the small number. Average water uptake of yolks from hens that had an average clutch size of one egg was significantly higher ($P<0.01$) than those from hens with a clutch size of 2, 3, or 4 eggs(9). No significant differences in water uptake were observed among yolks from the 2, 3, or 4 egg clutches.

After 6 days of daily administration of oil supplements, all the yolks tested from hens receiving *Sterculia foetida* oil (Exp. II, Lots 3 and 4) gave a positive Halphen test, whereas yolks from hens receiving corn oil (Lots 1 and 2) gave a negative test. The positive Halphen test indicated presence of sterculic acid(8) or a closely related compound in the yolk. Average water uptake of yolks and yolk weights of eggs from hens receiving corn oil or *Sterculia foetida* oil supplements is shown in Table II. After storage overnight, differences in water uptake and in yolk weights between eggs from corn oil and *Sterculia foetida* oil fed hens (Lots 1 and 3) were not significant. After storage of one month, significantly greater water uptake ($P<0.001$) was found in the eggs from the *Sterculia foetida* oil fed group (Lot 4). Yolks of eggs from the *Sterculia foetida* oil group (Lot 4) were 20% heavier and took up 80% more water than those from the corn oil group (Lot 2).

Discussion. Differences in water uptake between clutch sizes may be influenced by differences in yolk weights (Table I) because yolk weights of clutch size 1 were significantly greater than sizes 2, 3, or 4 ($P< 0.01$). Orru(6) reported higher water uptake values than those obtained in Exp. I. Orru(10) also reported that water uptake increased with storage time of the egg previous to incubation. Van Tienhoven, *et al.*(11) reported that average water uptake of fresh yolks was 1.268 g, but clutch size was not given. In our experience using Orru's(6) method of water uptake, the one egg clutch should be differentiated from the 2-4 egg clutch.

Increased water uptake by yolks from eggs in the *Sterculia foetida* oil lot occurs only after storage longer than overnight. Physical or chemical changes probably take place during storage of *Sterculia foetida* oil eggs which

TABLE II. Influence of Storage on Water Uptake and Yolk Weight of Egg Yolks Produced by Hens Fed *S. foetida* Oil or Corn Oil.

Lot	Supplement	Duration of storage at 7°C	Avg water uptake of yolks, g	Avg yolk wt, g
1	Corn oil	Overnight	.95 ± .29*(77)†	18.61 ± 1.07*(95)†
2	" "	1 mo	6.13 ± 2.07 (69)	19.36 ± 1.36 (89)
3	*S. foetida* oil	Overnight	1.03 ± .09 (31)	19.24 ± .09 (46)
4	" "	1 mo	11.12 ± 4.64 (11)	23.35 ± 1.74 (28)

* Mean ± stand. dev. † No. of observations.

do not occur during cold storage of corn oil eggs. This is supported by the observation that the putty-like yolks previously reported by Shenstone and Vickery(2) were produced only during cold storage of eggs from the *Sterculia foetida* oil lots. If eggs were stored at room temperature, this did not occur. Pink discoloration of eggs occurred only in eggs from Lot 4.

Summary. The yolks of eggs from hens having a one-egg clutch average took up significantly more water than those from hens having 2-4 egg clutch sizes. Yolks from hens having 2, 3, and 4 egg clutch sizes showed no significant differences in water uptake. Yolks from eggs stored overnight and from hens given *Sterculia foetida* oil were not significantly heavier and did not take up more water than eggs from hens given corn oil. However, when the eggs were stored one month the yolks from the hens fed *Sterculia foetida* oil increased 20% in weight and showed an 80% greater water uptake than those from the corn oil fed hens.

1. Masson, J. C., Vavich, M. G., Heywang, B. W., Kemmerer, A. R., *Science*, 1957, v126, 751.

2. Shenstone, F. S., Vickery, J. R., *Poultry Sci.*, 1959, v38, 1055.

3. Sherwood, R. M., *Texas Agr. Exp. Station Bull.*, 1928, v376, 5.

4. Schaible, P. J., Bandemer, S. L., *Poultry Sci.*, 1946, v25, 456.

5. Evans, R. J., Bandemer, S. L., Davidson, J. A., Bauer, D. H., *J. Agr. Food Chem.*, 1959, v7, 47.

6. Orru, A., *Arch. Sci. Biol.* (Italy), 1939, v25, 292.

7. Assn. Offic. Agr. Chemists, *Official Methods of Analysis*, 8th edition, Washington, D. C., 1955, 475.

8. Faure, P. K., *Nature*, 1956, v178, 372.

9. Li, J. C. R., *Introduction to Statistical Inference*, Edwards Bros., Inc., Mich., 1957.

10. Orru, A., *Riarca Sci.*, 1940, v11, 215, (C.A. 36:7165).

11. Van Tienhoven, A., Hill, F. W., Prock, A., Baker, R. C., *Poultry Sci.*, 1958, v37, 129.

Received July 29, 1960. P.S.E.B.M., 1960, v105.

Relation of Age to Radiomagnesium Exchange in Bone.* (26111)

SIDNEY BREIBART, JOHNG SUP LEE, AUGUSTA MCCOORD, AND GILBERT B. FORBES
Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Evidence suggests that the reactivity of the skeleton, as measured by isotope exchange, declines with age(1). Studies in our laboratory have shown that radiosodium exchange in young bone exceeds that of old bone in both the rat and cat(2). A direct relationship between liberation of magnesium from skeleton and rate of bone growth has been demonstrated by Duckworth and Godden(3). The observed depletion of bone magnesium in calves fed a magnesium deficient diet(4), together with the finding of a normal bone content in lactating cows with hypomagnesemia(5), also suggests that bone magnesium is more labile in young animals.

This report concerns exchange of radiomagnesium in the bones of rats of various ages. Data are also included on age changes in bone content of stable magnesium.

Methods. Magnesium^{28} (T½ 21 h.) obtained from Brookhaven National Laboratories, was supplied as a solution of $MgCl_2$ in concentrated HCl. Sodium hydroxide was added to bring the pH to about 5. Due to the short half-life of the isotope it was necessary to inject more than trace amounts of stable magnesium. The amount actually injected was in the range of 0.1 to 0.16 meq. Mg/100 g body weight, and average dose of Mg^{28} was $4x10^5$ c/m/100 g body weight.

Groups of female rats aged 20, 30, 60, 180 days were injected with measured amounts of the isotope intraperitoneally and after 4 hours were exsanguinated by decapitation. In some of the 180-day and 30-day animals,

* Supported by grants from U. S. Atomic Energy Comm., Contract with University of Rochester, and Nat. Inst. of Arthritis and Metab. Dis.