quantity for their carbohydrate and fat metabolism, it seems probable that glycerin protects the highly sensitive cell membranes from the damaging effect of heat at 45 °C.

1 H. Vollmar, Z. Krebsforsch. *51*, 71 (1941).
2 I. Fekete and P. Szabó, Naturwissenschaften *49*, 400 (1962).
3 I. Fekete, P. Szabó and I. Turi, Naturwissenschaften *50*, 156 (1963).
4 I. Fekete and P. Szabó, Naturwissenschaften *52*, 139 (1965).
5 M. v. Ardenne, Naturwissenschaften *52*, 419 (1965).
6 M. v. Ardenne, Naturwissenschaften *52*, 480 (1965).
7 M. v. Ardenne, Naturwissenschaften *52*, 645 (1965).
8 M. v. Ardenne and P.G. Reitnauer, Naturwissenschaften *53*, 88 (1966).
9 M. v. Ardenne, Naturwissenschaften *53*, 180 (1966).
10 M. v. Ardenne and W. Krüger, Naturwissenschaften *53*, 479 (1966).
11 M. v. Ardenne and W. Krüger, Naturwissenschaften *53*, 436 (1966).
12 M. v. Ardenne and W. Krüger, Z. Naturforsch. *21b*, 836 (1966).
13 M. v. Ardenne and P.G. Reitnauer, Z. Naturforsch *21b*, 841 (1966).
14 M. v. Ardenne, Naturwissenschaften *51*, 217 (1964).
15 M. v. Ardenne, Naturwissenschaften *51*, 415 (1964).
16 M. v. Ardenne and F. Rieger, Dte GesundhWes. *19*, 2136 (1964).
17 M. v. Ardenne and P.G. Reitnauer, Dte GesundhWes. *19*, 1355 (1964).
18 M. v. Ardenne and R. Tümmler, Dte GesundhWes. *19*, 709 (1964).
19 M. v. Ardenne, Dte GesundhWes. *19*, 49 (1964).
20 B. Mendel, Klin. Wschr. *8*, 169 (1929).
21 O. Warburg, K. Gawehn, A. Geissler and S. Lorenz, Z. klin. Chem. *1*, 175 (1963).
22 J.H. Riddick and R. Bressler, Biochem. Pharmac. *16*, 239 (1967).
23 H. Wand and G. Bacigalupo, Z. Naturforsch. *21b*, 1215 (1966).
24 J. Drews, Naturwissenschaften *51*, 515 (1964).
25 J. Drews and E. Fölsch, Naturwissenschaften *51*, 440 (1964).
26 W. Oelkers, M. Wetzel and P. Schmialek, Z. Naturforsch. *20b*, 227 (1965).

Hepatic α-tocopherol binding protein in the rat: Absence of an effect of selenium deficiency on binding activity

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Summary. Selenium deficiency produces no effect on either the total content of or the binding properties of rat liver α-tocopherol binding protein.

The biochemical basis for the interactions of selenium and vitamin E, first noted at the nutritional level, are slowly being understood. Following the discovery of Rotruck et al.[1] that selenium is a necessary component of the enzyme glutathione peroxidase, Hoekstra[2] suggested that glutathione peroxidase reduces lipid hydroperoxides while vitamin E prevents their formation, a proposal consistent with an interaction of these nutrients in oxidant-induced disease. Other observations, however, suggest interactions not explained on this basis. For example, a number of laboratories have reported that at marginal vitamin E intakes, increased dietary selenium results in a decrease in plasma tocopherol levels in rats[3-5]. Other studies[6] have shown that at adequate vitamin E intakes the converse relationship exists, i.e. with increased dietary selenium there is an increased plasma tocopherol. Either situation suggests that selenium affects the absorption, transport or utilization of tocopherol.

We have recently described a highly specific α-tocopherol binding protein in experimental animals that is detectable only in the liver[7]. This localization, coupled with the fact that liver necrosis results from a combined selenium and vitamin E deficiency in rats, suggested that a possible interaction should be investigated. Accordingly, a study was undertaken to examine the effects of selenium deficiency on the binding of α-tocopherol to its hepatic binding protein.

Methods and materials. Female Fisher strain rats with 1-week-old litters were gradually introduced over a 4-day period to a selenium and vitamin E-deficient diet based on torula yeast as previously described[8]. The male pups were weaned at 21 days, caged individually, and divided into 3 dietary groups. The 1st group continued to receive the basal, doubly deficient diet; a 2nd group received basal diet supplemented with 0.1 ppm selenium as sodium selenite, and a 3rd group received a vitamin E-deficient, selenium-sufficient casein diet[9]. Rats were allowed free access to the diets and water.

At approximately 18 days post weaning, rats receiving the basal torula yeast diet began dying from liver necrosis. At this time 3 rats from each group were sacrificed and liver cytosol preparations made for assay of α-tocopherol binding protein as previously described[7]. Only the non-necrotic portions of the livers of rats receiving the doubly deficient diet were used. Protein content of the cytosol was measured by Biuret[10].

Results and discussion. Initial studies of the α-tocopherol binding protein were made with livers from rats fed the vitamin E-deficient, selenium-adequate casein diet. It was shown that supplemental dietary tocopherol reduced the

Effects of selenium deficiency on the specific activity of rat liver α-tocopherol binding protein

Diet	Body weight (g)	Liver weight (g)	Cytosol* protein (mg/ml)	Tocopherol bound pmoles/mg cytosol protein	pmoles/g liver
Torula yeast	50.3±0.6	2.6±0.1	7.8±0.6	3.3±0.2	126.7±16.6
Torula yeast +selenium	69.0±4.0	5.2±0.3	7.2±0.5	3.2±0.2	114.3± 8.3
Casein	77.0±2.0	4.9±0.2	12.9±0.8	3.1±0.1	207.3±22.2

Values are mean±SD. * Cytosol = 105,000×g supernatant. Tissues were homogenized in 4 volumes of buffer.

observed binding of [3H] α-tocopherol to its hepatic binding protein, presumably through competition from endogenous tocopherol[11]. Thereafter animals fed the vitamin E-deficient diet were used to establish optimal assay conditions and properties of tocopherol binding protein[7]. In the present experiment, an experimental group fed this diet was also included as a basis for comparing binding activity with different dietary treatments.

The table illustrates that selenium deficiency produced no effect on the specific activity of α-tocopherol binding protein whether activity was calculated as pmoles/mg protein or as pmoles/g tissue. The only significant difference observed was in the content (pmoles/g liver) of binding protein in the liver of animals fed the casein diet. This higher activity probably results solely from an increase in total soluble liver protein concentration (12.9 mg/ml vs 7.8 and 7.2 mg/ml) rather than an effect of selenium. It should be noted that the torula yeast diet is considerably lower in protein (15%) than the casein diet (22%).

These data suggest that either the binding protein is not a seleno-protein or selenium, if present, is not necessary for optimal binding. We cannot eliminate, however, the possibility that because of the low estimated concentration of the binding protein in liver (approximately 200 pmoles/g)[7], traces of selenium could maintain activity. In deficiency produced by a similar diet, liver selenium levels of 70 ng/g (800 pmoles/g) have been reported[3,12] representing more than a sufficient amount of selenium on a molar basis to serve functionally in the binding protein. It does, however, seem unlikely that depletion of selenium from normal levels of 750 ng/g to 70 ng/g (Burk et al.[3]), over a 10fold decrease, would be entirely without effect on the binding protein, were it a seleno-protein.

Although the function of the hepatic α-tocopherol binding protein is at present unknown, it appears that its binding properties are unrelated to the selenium status of the animal. This system therefore seems unattractive for further study of a functional interaction between selenium and tocopherol.

1 J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, Science *179*, 588 (1973).
2 W.G. Hoekstra, Fedn Proc. *34*, 2083 (1975).
3 R.F. Burk, J, R. Whitney, H. Frank and W.N. Pearson, J. Nutr. *95*, 420 (1968).
4 P.D. Whanger and P.H. Weswig, Nutr. Rep. Intern. *12*, 345 (1975).
5 P.R. Cheeke and J.E. Oldfield, Can. J. Anim. Sci. *49*, 169 (1969).
6 O.L. Scott, J. Kelleher and M.S. Losowsky, Biochim. biophys. Acta *497*, 218 (1977).
7 G.L. Catignani and J.G. Bieri, Biochim. biophys. Acta *497*, 349 (1977).
8 J.G. Bieri and K. Ahmad, J. Agric. Food Chem. *24*, 1073 (1976).
9 R.P. Evarts and J.G. Bieri, Lipids *9*, 860 (1974).
10 E. Layne, in: Methods in Enzymology, vol. 3, p. 450. Academic Press, New York 1957.
11 G.L. Catignani, Biochem. biophys. Res. Commun. *67*, 66 (1975).
12 H.D. Hurt, E.E. Cary and W.J. Visek, J. Nutr. *101*, 761 (1971).

Alpha-globulin follicular fluid proteins within small and large bovine follicles[1]

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Summary. Fluids from small (FF-S) and large (FF-L) bovine ovarian follicles were observed electrophoretically for differences in the alpha-globulin protein profile. FF-L possessed a fast migrating alpha-globulin 3 and a greater percentage of FF-L samples contained a higher number of alpha-globulin bands.

Protein components of bovine ovarian follicular fluids (FF) have been investigated mainly to dermine whether the proteins resulted from filtration of the blood or were products of granulosa and theca cells[2,3]. In these studies the fluids aspirated from all follicles were pooled for the determinations and no attempt was made to investigate the possible qualitative protein differences in fluids from follicles of varying sizes. The electrophoretic examination of porcine FF indicated differences in the alpha-globulin profile between fluids from small and large follicles[4]. The intrafollicular role of the alpha-globulins is unknown, but in the pig these proteins may control the development of follicular oocytes through steroid transporting function or by acting as inductor or repressor substances[4]. The purpose of this study was to investigate the electrophoretic properties of the alpha-globulin proteins within bovine follicular fluids of individual small and large intact follicles.

Materials and methods. Blood and ovaries from 18 cows were obtained at a local abattoir. The ovaries (each pair bearing a corpus luteum-CL) were placed in iced physiological saline, and within 3 h of slaughter the diameters of the ovarian follicles were obtained. An arbitrary scale was used to distinguish between fluids from small follicles (FF-S; 2.0-5.0 mm) and large follicles (FF-L; 5.1-15.0 mm). Fluids were aspirated from individual follicles from each female and kept separate. Blood samples were centrifuged at 2100×g for 20 min with serum collected and frozen. Protein concentration was quantitated for each serum and FF sample using bovine serum albumin as the standard[5]. Serum progesterone was quantitated by radioimmunoassay[6] to assess CL-function. The specificity of the antisera to progesterone has been characterized[7].

A 'freeze-thaw' and centrifugation procedure was used to remove fibrinogen from each serum and FF sample[8]. Protein quantitation was repeated and 250 µg of protein from each serum, FF-S and FF-L sample were subjected to polyacrylamide gel-electrophoresis[6]. Migration was toward the anode using Tris-borate buffer (0.03 M; pH 9.0). Gels were stained with 0.2% Coomassie blue G and destained in methanol:H_2O:acetic acid (45:45:10 v/v). The number of stained alpha-globulin bands and the mobility[9] of these bands plus the albumin band was recorded for each serum, FF-S and FF-L sample. Protein (mg/ml) and mobility data for serum FF-S and FF-L were analyzed by the Newman-Keuls test and differences in the percentage as related to number of alpha-globulin bands among treatments were analyzed by Chi-square[10].