lysozyme differ biochemically. However, the relationship between these two substances will not be clarified definitely until both are characterized in precise chemical terms.

Summary. Endogenous pyrogen (EP) and lysozyme concentrations in serum paralleled one another when dogs were challenged with varying doses of endotoxin, were made tolerant to the action of the toxin, and were given endotoxin along with aminopyrine, adrenal steroids, and hypothermia. Despite these biologic parallels, there is no evidence that endogenous pyrogen and lysozyme are the same biochemical substance.

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Selenium and Vitamin E Influence upon the *in vitro* Uptake of Se^{75} By Ovine Blood Cells.* (28683)

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Incorporation of selenium into circulating blood cells following administration of radioselenium has been reported in swine(1), in the dog(2), in sheep(3) and in the chick(4). McConnell(2) has demonstrated that selenohemoglobin is produced following radioselenate administration.

The mechanism through which selenium enters the blood cell is not known, although the accumulation and retention of Se^{75} following a single dose is dependent upon dietary selenium intake in the chick(4). While it is well documented that active transport mechanisms are present in the erythrocyte membrane for both sodium and potassium, it has not been determined from the *in vivo* experiments if such is the case for selenium.

These experiments were conducted to determine if the uptake of selenium by intact ovine blood cells *in vitro* would reflect the dietary intake of selenium. Sulfur, cesium, and zinc were studied to determine if the differences observed for selenium were specific for selenium or indicative of increased membrane permeability in the cells from the animals on the low selenium diets. In view of the observed interrelationships of selenium and Vit. E in prevention of certain pathological changes(5), the effects of dietary α-tocopherol, both with and without supplemental selenium, were also investigated in this system.

Methods and materials. The basal ration used in these experiments is shown in Table I. The mineral mixture, essentially that of

TABLE I. Percentage Composition of Basal Diet.

Cellulose*	30.0	Stripped lard†	2.0
Corn starch	29.7	Choline chloride	.1
Cerelose	29.7	Ground plastic‡	1.0
Urea	4.0	Mineral mixture§	3.5

* Solka-floc, BW-40. Brown Co., New York.
† Molecular distillation, gift of Distillation Prod. Ind., Rochester, N. Y.
‡ Polyethylene, M-700. E. I. du Pont de Nemours Co., Wilmington, Del.
§ Percent: $CaHPO_4$, 48.84; K_2CO_3, 31.55; $MgSO_4$, 10.75; NaCl, 7.43; $FeSO_4$, .91; $Na_2B_4O_7$, .19; $ZnSO_4$, .14; $MnSO_4$, .10; KI, .03; $CuCO_3$, .02; MoO_3, .0008; $CoCl_2$, .0003.

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Oltjen *et al.*(6), consisted of reagent grade chemicals. The basal diet contained 0.015 ppm selenium as determined by neutron activation analysis at Oak Ridge Nat. Laboratory. Vit. D_2 was added to the diet at a level that would provide 400 IU per ewe per day. Vit. A was injected intramuscularly at weekly intervals to provide 2500 IU per ewe per day. The animals were housed in a barn, open on one side, with concrete floors. No bedding was provided.

In the first trial 6 mature ewes which had been fed their experimental ration for the preceding 10 months were used. Each of these ewes were given 100 IU of Vit. E per day as a drench, and one-half of them received 0.5 ppm selenium as sodium selenite in the ration. Six additional ewes received a natural ration of pasture, hay, and grain.

Freshly drawn heparinized whole blood was used in these experiments. Ten-ml aliquots of blood in 25-ml Erlenmeyer flasks were incubated with 60 millimicrocuries (mμc) Se^{75} as H_2SeO_3 (>5000 mc/g Se), or of Zn^{65} as $ZnCl_2$ (>75 mc/g Zn), or of Cs^{134} as CsCl (>3000 mc/g Cs) contained in 25 λ of saline. One hundred and twenty mμc of S^{35} as Na_2SO_4 (carrier free) was used. Radioisotopes were obtained from the Oak Ridge National Laboratory. The flasks were shaken during incubation, temperature was maintained at 38°, and the atmosphere was either 95% oxygen, 5% carbon dioxide or 95% nitrogen, 5% carbon dioxide (Welco Medical Gas). At varying times during the incubation, 1 ml samples were drawn directly from the incubation flasks and placed into plastic scintillation tubes. The cells were then washed twice with 20 volumes of cold isotonic saline; refrigeration was maintained during centrifugation. Gamma counting was carried out in the washing tubes in a well-type scintillation counter. After the cells incubated with S^{35} were washed, they were transferred directly to glass scintillation vials and prepared for liquid scintillation counting (7). Packed cell volumes (pcv), determined at the times counting samples were taken, remained constant throughout the incubations. Percentage uptake of radioactivity in all samples was corrected to a pcv of 0.40.

In the second trial 26 nine-month-old ewe lambs were placed on the experimental ration (Table I) at time of mating. These ewes were divided into 4 groups, representing a factorial arrangement of the above levels of Vit. E and selenium (7 received Se + E, 7 received E, 6 received Se, and 6 received no supplementation). Blood samples were collected from these ewes at 3-week intervals and incubated with Se^{75} for 1 hour under a nitrogen-carbon dioxide atmosphere as described above.

Statistical significance of the data was determined by analysis of variance.

Results. In Fig. 1 and 2 are presented the cellular uptake of Se^{75} when the incubations were carried out in presence of oxygen and nitrogen, respectively. An increase in uptake of selenium with time was found regardless of treatment when the cells were incubated under an atmosphere of oxygen. Cells from ewes receiving the basal ration retained approximately twice ($P < 0.01$) as much activity as cells from ewes receiving either the natural ration or the 0.5 ppm selenium-supplemented ration. When the incubation was conducted in the presence of nitrogen, there was no increase in uptake of Se^{75} beyond the initial influx. The relative differences among dietary groups were maintained. Although the values from ewes on the natural ration averaged higher than the values for the 0.5 ppm selenium-supplemented ewes, these differences were not statistically significant.

The stroma, from cells lysed with water following incubation, contained only a trace of the total Se^{75}.

No differences were observed either in rate or amount of cellular uptake of zinc, cesium, or sulfur which could be attributed to dietary selenium level (Fig. 3, 4, and 5). Neither was the rate of influx of these materials oxygen dependent.

The results of the second trial, involving 26 ewes, are shown in Fig. 6. When individual blood samples were incubated with Se^{75} for 1 hour in the presence of nitrogen, no significant differences were observed among treatments until the sixth week on the experimental ration. At this time the cells from ewes receiving 0.5 ppm selenium took

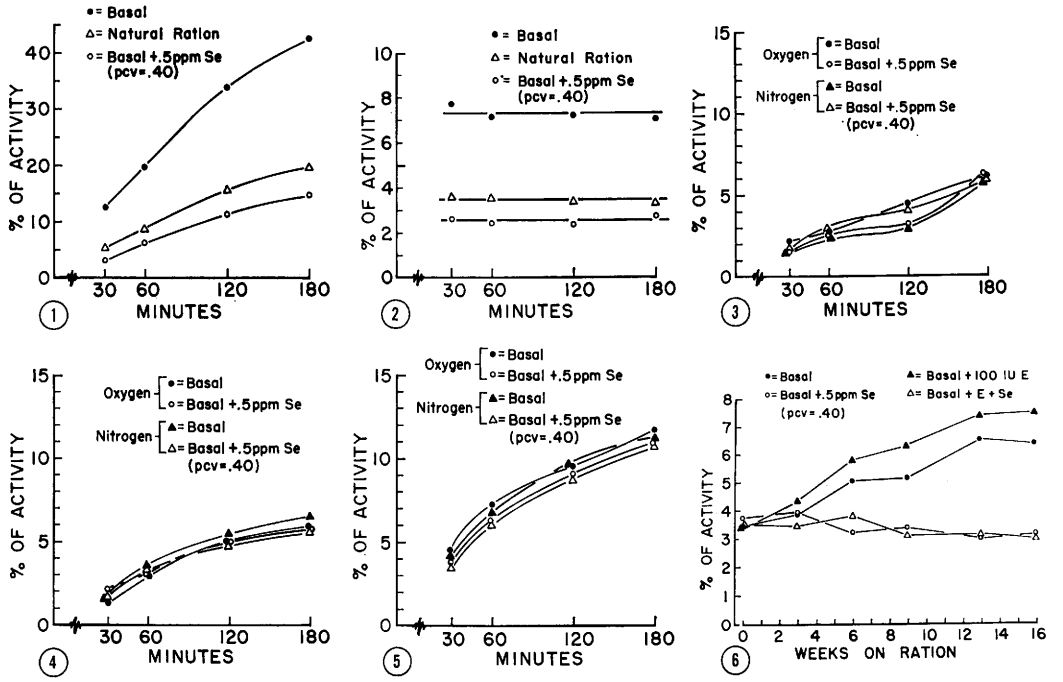

FIG. 1. Influence of diet upon *in vitro* uptake of Se-75 by ovine blood cells incubated under oxygen.

FIG. 2. Influence of diet upon *in vitro* uptake of Se-75 by ovine blood cells incubated under nitrogen.

FIG. 3. Influence of dietary selenium upon *in vitro* uptake of Zn-65 by ovine blood cells.

FIG. 4. Influence of dietary selenium upon *in vitro* uptake of Cs-134 by ovine blood cells.

FIG. 5. Influence of dietary selenium upon *in vitro* uptake of S-35 by ovine blood cells.

FIG. 6. Effect of dietary selenium and α-tocopherol upon *in vitro* uptake of Se-75 by ovine blood cells.

up less Se^{75} ($P < 0.05$) than cells from ewes on the basal ration. The differences between these groups increased ($P < 0.01$) during the remainder of the 16-week experiment. The differences in the uptake by cells from ewes receiving the basal ration plus Vit. E and ewes receiving only the basal ration were not statistically significant.

Complete hematological analysis of blood samples from each of the experimental animals revealed no quantitative or morphological cellular differences attributable to dietary treatment.

Discussion. The *in vitro* uptake of Se^{75} by ovine blood cells was inversely proportional to the dietary intake of selenium. Approximately 6 weeks elapsed before changes in dietary selenium level were reflected in uptake of Se^{75} by blood cells. These differences increased during the remainder of the experimental period. The period required for these differences to become apparent may be indicative of the length of time required for an equilibrium between dietary intake, body stores, and excretion to be reached in the sheep.

Vit. E supplementation did not markedly influence Se^{75} retention by the blood cells *in vitro*. Jensen *et al*(4) reported a similar lack of effect of Vit. E upon selenium distribution in various tissues of the chick *in vivo*.

Differences in selenium influx when blood was incubated in the presence of oxygen, as opposed to nitrogen, indicate that a mechanism requiring active respiratory processes by the cell membrane is required for *in vitro* accumulation of selenite by the ovine blood cell. This may involve an oxidation of selenite to selenate. It is suggested that the limited influx of selenium in the presence of nitrogen takes place prior to the decline in

oxygen concentration below the level which would support cellular respiration. Following the initial influx no increase in cellular uptake is observed in the absence of oxygen, indicating that a passive diffusion of selenite into the cell does not occur. The ability of cyanide(8) to inhibit the uptake of selenium both in the presence of oxygen and of nitrogen supports this hypothesis.

When blood from these same sheep was incubated with zinc or cesium, no differences in cellular uptake were found, indicating that the increased uptake of selenium by cells of the selenium-deficient ewes was specific and not due to a simple increase in cellular permeability.

Similarly the *in vitro* uptake of sulfur was not influenced by dietary selenium intake. This would agree with the hypothesis(9) that at physiological levels selenium and sulfur do not follow identical metabolic pathways.

The convenience with which this technique can be conducted and the sensitivity and reproducibility it offers suggest its value as an assay of the dietary level of selenium of animals in the field and as an indication of areas(10) where selenium supplementation would prove beneficial.

Summary. Whole blood from ewes being fed a purified diet containing urea as the sole nitrogen source was used to determine the *in vitro* uptake of selenium by ovine blood cells. Selenium-^{75} as selenite moved into the ovine blood cell *in vitro* via an oxygen-dependent transport mechanism. The magnitude of this influx was inversely proportional to the dietary intake of selenium, but was not affected by dietary Vit. E. The uptake of sulfur, zinc, or cesium was not influenced by dietary selenium level.

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Calcium Metabolism in the Rat Studied with Calcium^{45}: Effect of Age. (28684)

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We have undertaken a series of investigations directed toward establishing the main parameters of calcium metabolism in rats in a variety of experimental states; the present paper deals with the effect of age, which is considered to have a profound effect on calcium metabolism(1,2,3).

Previous investigations have indicated that with advance in age there is an initial increase in bone density(4) which, in old age, remains constant in rats or decreases in man (5), an increase in the calcium-pyrophosphate ratio(6), alteration in intestinal absorption of calcium (decreased — 1, 7, 8; increased — 9), reduced calcium retention(7), changes in the kinetics of Ca^{45} specific activity of tissues(10) and a decline in endochondral osteogenesis(11). The majority of these studies were mainly concerned with senescence. The present report concerns the differences in calcium metabolism between young actively growing rats and adult animals at the growth plateau.

Material and methods. Two groups of