

Acta vet. scand. 1973, 14, 219—224.

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## THE DISTRIBUTION OF $^{35}\text{S}$ IN THE ACUTE INVOLUTION OF THE CANINE THYMUS

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OKSANEN, AILI: *The distribution of  $^{35}\text{S}$  in the acute involution of the canine thymus.* Acta vet. scand. 1973, 14, 219—224. — Sulphur metabolism was studied in thymuses of 8 young dogs stressed by peripheral irradiation. Autoradiographic studies revealed a significantly heavier uptake of  $^{35}\text{S}$  in the medulla, rich in reticular cells, than in the cortex ( $t = 9.556$ ,  $P = 0.001$ ). In epithelial cysts in the thymus the uptake of  $^{35}\text{S}$  was so dense that it appeared as black areas. Possibility of sulphur-containing secretion was discussed.

thymus; reticular cells; epithelial cysts;  $^{35}\text{S}$ ;  
irradiation.

In the epithelial cells of the thymus or in the contents of the cysts sometimes formed by these, sulphur-containing substance has been demonstrated by the Alcian blue method in mice (*Clark* 1966), rats (*Oksanen* 1966), dogs (*Oksanen* 1968) and snakes (*Oksanen & Tuurala* 1970). In mice,  $^{35}\text{S}$  first enters the reticular cells (*Clark*). By the aid of  $^{35}\text{S}$ , it was shown that irradiation of chicks did not depress the proliferative capacity of the thymus epithelium (*Khussar* 1964). In a  $^{35}\text{S}$  study in the rat by *Diderholm & Hellman* (1958), the autoradiogram of the thymus in one instance showed a black area indicating a massive uptake of  $^{35}\text{S}$ . On haematoxylin staining this area proved to be a ciliary epithelial cyst.

So far only 3 autoradiographic  $^{35}\text{S}$  investigations on the thymus seem to have been published. Since the involution of the thymus caused by irradiation activates the reticular cells of this organ (*Khussar*) and increases the incidence of thymus cysts even in young dogs (*Oksanen 1968*), it was considered useful to study the distribution of  $^{35}\text{S}$  in the acute thymus involution caused by irradiation.

### MATERIAL AND METHODS

The series consisted of eight healthy young mongrel dogs. The dogs received peripheral irradiation to joints. A 3000 Ci Tele-Cobalt unit (FSD 75 cm, dose rate 104/rad/min.) was used. Four adult dogs were given 5000 rad, and four puppies 3000 rad. In both age groups 2 dogs were killed with T 61 4 days, the remainder 14 days, after irradiation. For autoradiographic examination  $^{35}\text{S}$  was intraperitoneally injected 24 hrs. before sacrifice. The  $^{35}\text{S}$  was given as sulphate in aqueous solution, pH 6–8, carrier-free A.1.D.6, and the dose was 500  $\mu$  Ci per kg of body weight. The injection time was the same throughout in order to guarantee similar effects.

Immediately after sacrifice, histological specimens of the thymus were taken from 2 sites. The specimens were fixed in neutral formalin (10 %), mounted in paraffin and sectioned at 5  $\mu\text{m}$ .

The specimens were mounted on glass slides and coated with Kodak NTB emulsion. The slides were placed in light-tight boxes with calcium chloride as dehydrating agent and stored for 3 months at 4°C. They were then developed in Kodak D 11 developer and fixed with Kodak rapid fixer, after which the sections were stained with haematoxylin-eosin.

Grain counts were made using an oil immersion objective, with an  $\times 400$  objective and  $\times 8$  ocular. Using a chequered measuring ocular the grains were counted in 10 adjoining areas, measuring 80  $\mu \times 8 \mu$ , from 2 random sites in both the lymphocyte-rich cortex and in medulla rich in reticular cells.

The mean values of grains in fields and their standard deviations were calculated for cortex and medulla, and Student's t-test was used on comparing them (*Fischer 1950*). Serial sections were stained by the PAS and Aloian blue techniques.

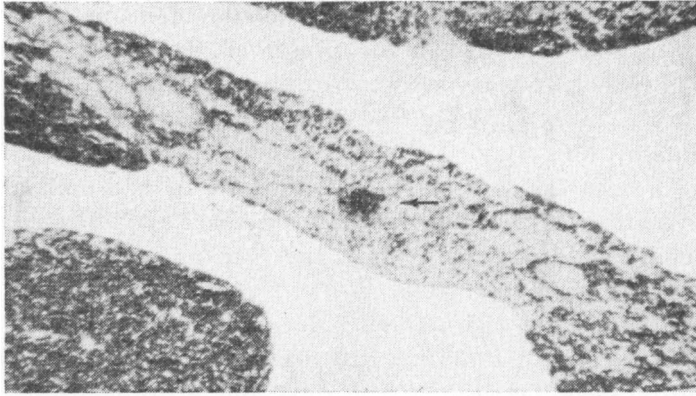


Figure 1. Involved thymus lobulus of a dog. An epithelial cyst filled with labelled <sup>35</sup>S. Haematoxylin and eosin. × 106.

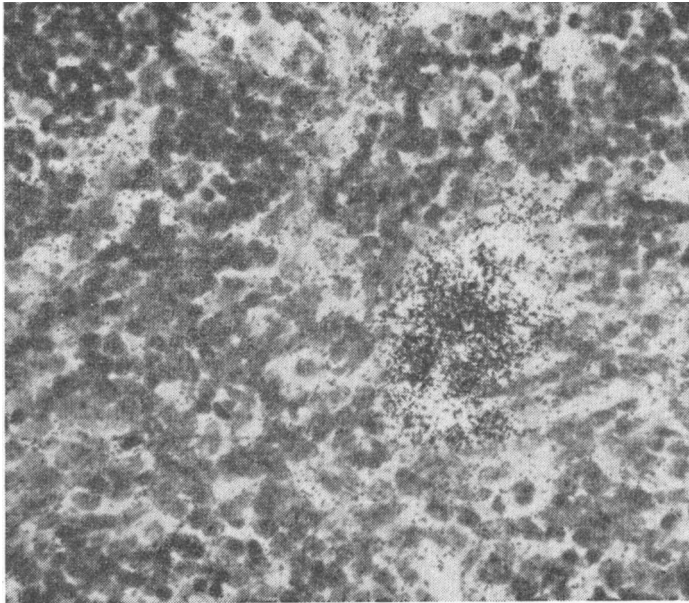


Figure 2. Two thymus cysts with grains of <sup>35</sup>S. The surrounding area is rich in reticulum cells mixed with lymphocytes. Haematoxylin and eosin. × 420.

## RESULTS

On histological investigation the lobular division of the thymus was mostly found to be preserved. However, involution varying in degree was seen in areas in the form of thinning of the cortex. Cilial epithelial cysts were observed in 3 dogs. The contents of the cysts and the cilia stained pale blue with Alcian blue. These areas were also PAS-positive. In addition, a PAS-positive granular reaction was observed in the cytoplasm of the epithelial cells lining the walls of the cysts and in the cytoplasm of some large reticular cells.

The mean and the standard deviation of the grain counts were in the medulla  $133.7 \pm 72.3$  and in the cortex  $71.3 \pm 39.7$ . The differences between the cortical and medullary uptake of  $^{35}\text{S}$  as demonstrated by Student's t-test was highly significant ( $t = 9.556$ ,  $P = 0.001^{***}$ ; d.f. = 14). The silver granulation of the epithelial cysts observed in 3 dogs was so dense that counting was impossible (Figs. 1 and 2).

## DISCUSSION

The present experiments showed that the uptake of  $^{35}\text{S}$  was highly significantly stronger in the medulla rich in reticular cells than in the cortex with its predominance of lymphocytes. This is in contrast to the observation that 24 hrs. after the injection of  $^{35}\text{S}$  the uptake was evenly distributed over the whole area of the thymus (Clark 1966). Furthermore, in the present experiments a very dense uptake of  $^{35}\text{S}$  was observed in epithelial cysts in the thymus. This confirms the observation mentioned in the foregoing that uptake of  $^{35}\text{S}$  was reflected as a dark area in an epithelial cyst in the rat thymus 3 weeks after injection of  $^{35}\text{S}$  (Diderholm & Hellman 1958). In the present study sulphur was found to enter such cysts much more rapidly, even within 24 hrs., in connection with the involution of the thymus, which has been found to activate the reticular cells in it (Khussar 1964) and to raise the incidence of thymus cysts (Oksanen 1968).

The abundant radioactive uptake in the reticular epithelial cells seems to indicate that the metabolism of the substance in question in these cells is intensive, owing to either mitosis, growth or secretion. The abundant accumulation of  $^{35}\text{S}$  within 24 hrs. not only in the reticular cells but also in epithelial cysts in the

thymus may be evidence of a sulphur-containing secretion. The possibility of a thymus secretion has been suggested by many earlier investigators both on the basis of biochemical studies of thymus extracts (*Szent-Györgyi et al.* 1962, *Comsa* 1965, *Pansky et al.* 1965) and the morphology of the reticular epithelial cells and cells lining the cysts (*ver Eecke* 1899, *Arnesen* 1958, *Clark*, *Kostowiecki* 1967, *van Haelst* 1967, *Glucksmann & Cherry* 1968, *Oksanen & Tuurala* 1970).

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## SAMMANFATTNING

*Fördelning av <sup>35</sup>S i hundthymus vid akut involution.*

Svavelomsättningen in thymus från 8 unga hundar, vilka stressats med perifer bestrålning, har undersökts. Autoradiografiskt kunde visas starkare ansamling av <sup>35</sup>S i medullan, som är rik på retikulära celler, än i cortex. I thymus' epiteliäl cystor var <sup>35</sup>S ansamlingen så stark, att denna sågs som svara fläckar. En eventuell svavehaltig thymussekretion diskuteras.

*(Received January 12, 1972).*

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