phyrin suggest that the porphyrinogens supplied in these experiments serve as less satisfactory substrates than the colorless enzymatic product for the production of porphyrins with fewer than eight carboxyl groups per molecule. It thus appears doubtful that the enzymatic product is identical with the bulk of the porphyrinogen produced by the palladium-hydrogen reduction of uroporphyrins. Inquiry into the nature of the enzymatic product is being continued (*5*).

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4 May 1955.

On the Supposed Contamination of Thymus Nuclear Fractions by Whole Cells

In a recent communication Brown (*1*) has claimed that thymus nuclear fractions prepared in sucrose-$CaCl_2$ solutions (*2*) are made up in large part of intact thymocytes. It should be emphasized that this claim is not based on the visible demonstration of the intact cells but rests rather on some differences in osmotic behavior shown by individual components of a nuclear suspension. Because these differences in osmotic behavior did not fit certain hypothetical expectations, they were taken to indicate a distinction between isolated cell nuclei and intact cells, and the conclusion was drawn that small thymocytes comprise a considerable proportion of the isolated "nuclear fraction." It was admitted, however, that such thymocytes could not be seen under ordinary or phase-contrast microscopy.

The difficulties in establishing the purity of nuclear preparations by light microscopy alone have concerned workers in this laboratory for some time. It is the purpose of this communication to demonstrate that thymus nuclei prepared in 0.25*M* sucrose-0.0018*M* $CaCl_2$ solutions are contaminated by thymocytes to only a slight extent. This demonstration rests on the observation and examination of the isolated nuclei under the electron microscope.

The appearance of thymus nuclei, isolated as previously described (*2*), is shown by the electron micrographs in Fig. 1 (*3*). Only one thymocyte (indicated by the arrow in the bottom photograph) can be detected in these two fields. The proportion of such intact cells in microscope fields selected at random is very low; of the order of 29 small thymocytes per 1000 nuclei. A careful scrutiny of electron micrographs shows that whole cell contamination is not a problem in such nuclear preparations. (It is realized that details of structure that are strikingly obvious in the original electron micrograph may not be as evident when the plates are reduced to the small size of Fig. 1.)

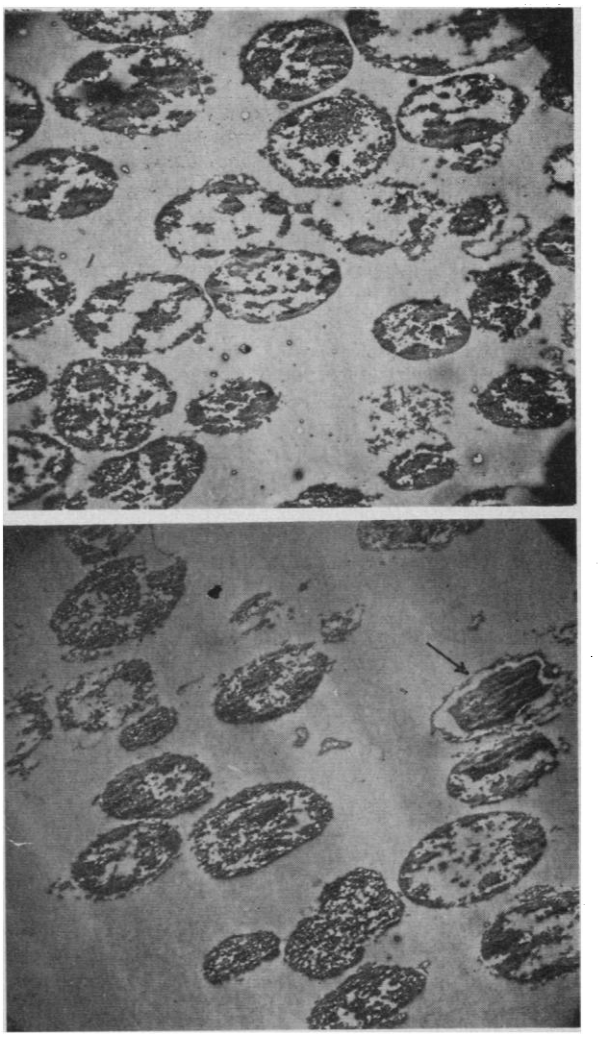

Fig. 1. Electron micrographs of thin sections of calf thymus nuclei following isolation in 0.25*M* sucrose-0.0018*M* $CaCl_2$ solution. A thymocyte is indicated by the arrow in the bottom photograph. (× 10,000)

What is evident is that thymus nuclei prepared in sucrose-$CaCl_2$ solutions are not entirely free of small amounts of adhering cytoplasm. This type of contamination is more difficult to evaluate. Its extent

can be roughly estimated by microscopic observation and, more accurately, by nucleic acid analyses of the nuclei and of the tissue. It is probably below 10 percent.

The reasons for the differences in osmotic behavior reported by Brown are not known to us, but we have had wide experience with varying degrees of nuclear fragility depending upon such variables as age of thymus, concentration of Ca^{++} or Mg^{++} ions, the molarity of the sucrose solution, temperature, shaking, and so forth. We do not feel that such differences by themselves should be taken to show either the presence or the absence of whole cells, especially when they lead to conclusions that are not in accord with direct observations by light or electron microscopy.

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14 April 1955.

Skin Symptoms of Vitamin-B_6 Deficiency in the Dog

About 20 years ago György (*1*) named the "rat antipellagra factor" vitamin B_6 and selected skin symptoms in the rat as the basis of a biological method of assay (*2*). He and his coworkers (*3*) referred to the dermatitis as acrodynia. It affects the peripheral parts of the body—nose, mouth, ears, paws, and tail. Affections of the skin and hair in vitamin-B_6 deficiency have been seen in other species—notably in the hamster (*4*), monkey (*5*), and pig (*6*)—but they do not show close similarity to one another or to the dermatitis that occurs in the rat.

In 1938 György (*7*) suggested the possibility of a relationship between insufficiency of vitamin B_6 and certain skin lesions in man, and in 1940 Smith and Martin (*8*) reported the successful treatment of human chielosis with pyridoxine. More recently Vilter and his associates (*9, 10*) reported the occurrence of seborrheic dermatitis about the eyes, nose, and mouth of human subjects given desoxypyridoxine, a compound usually regarded as a B_6 antivitamin. An ointment containing pyridoxine alleviated the seborrhea and was also effective in some other cases of dermatitis (*10*).

For a number of years I have been conducting experiments on vitamin-B_6 deficiency in rats and dogs. In the former I have induced the recognized effects on growth and metabolism, and in the latter, the typical hematological symptoms. In only a small number out of hundreds of such rats have I seen acrodynia, and then only after a period of months on the deficiency regimen. This is at variance with the impression given by the reading of a standard textbook treatment of vitamin-B_6 deficiency. If desoxypyridoxine is given, however, rats usually develop skin symptoms in a relatively short time that are indistinguishable from those of simple vitamin-B_6 deficiency. In dogs deprived of vitamin B_6, I have never seen effects on the skin or hair, even with severe hematological symptoms. However, in a few young dogs that have been given desoxypyridoxine, I have seen rather severe skin lesions that seem to resemble those which Vilter and his associates induced in human subjects. This has always been associated with the feeding of desoxypyridoxine in a diet containing 40 percent casein, it has occurred only in pups a few months of age, and only in three out of nine that have been studied on this particular regimen.

Since no description of this effect appears to have been published, I report it here, for it may be of interest to those who are concerned with vitamin B_6 and the health of the skin. Any conclusions of nutritional significance from such observations should, however, be made with caution. Desoxypyridoxine is effective in much smaller amounts than other so-called "antivitamins" (*11*), and it has a quick and profound effect on an animal. The question still remains whether its action is entirely the result of metabolic interference with vitamin B_6.

In these dogs the eyes are particularly affected, and the animal appears to be conscious of soreness in them. There is a conjunctivitis with mucous discharge, blepharitis, and a "spectacle-eye" effect from denudation. The skin about the eyes, nose, and mouth shows a pink coloration. These symptoms rapidly improve upon withdrawal of desoxypyridoxine and with the administration of a small amount of pyridoxine, but the denudation about the eyes is rather persistent.

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