Short Communications

Tocopherol Levels in Normal Pigs
and in Pigs with Waxy Muscle
Degeneration and *Hepatosis*
diaetetica

HANS WANNTORP and ANNA-LISA OBEL

*Departments of Chemistry and Pathology,
State Veterinary Medical Institute,
Stockholm, Sweden*

Vitamin E or tocopherol was first considered to be a fertility or antisterility vitamin on the basis of early experiments with rats. Numerous investigations have since demonstrated, however, that this vitamin has a much wider range of activity. In animals other than rats including domestic animals, the major activity seems to be as an antioxidant in metabolic processes.

The most commonly used pig feeds are relatively deficient in vitamin E. It is, therefore, to be expected that lesions often occur in pigs which may be the expression of a deficiency of this vitamin. Many of the changes which can be observed in experimental vitamin E deficiency in the rat such as waxy degeneration of the muscles, ulceration in the cutaneous partition of the mucous membrane of the stomach, and so-called yellow fat can also be seen in many pig autopsies. Vitamin E deficiency is also an aetiological factor contributing towards the production of the *hepatosis diaetetica* syndrome. Obel¹ was able to produce this disease experimentally through a deficiency of vitamin E and sulphur-containing amino acids.

In order to establish whether or not the tocopherol level in the tissues of pigs with waxy degeneration of the muscles and *hepatosis diaetetica* is lower than is the case for

healthy animals, determinations were carried out for various groups of animals. The tocopherol content of the heart, liver, and spleen was determined by the method of Wanntorp and Nordlund².

The average values for tocopherol in the heart, liver, and spleen of normal slaughter pigs (group 1) was 1.42, 0.96, and 0.59 mg/100 g, respectively. Group 2 consisted of pigs submitted for autopsy and which were affected by diseases other than those which could be associated with vitamin E deficiency. Average values for the hearts, livers, and spleens from this group were 1.07, 0.67, and 0.45 mg/100 g, respectively. Both these groups can be taken as comparison material for the other groups which consisted of animals probably deficient in vitamin E, *i. e.*, pigs with waxy degeneration of the skeletal musculature, group 3, pigs with *hepatosis diaetetica*, group 4, and pigs with experimental vitamin E deficiency, group 5. The average values obtained for tocopherol in group 3 were 0.76, 0.52, and 0.34 mg/100 g, respectively, in group 4, 0.59, 0.38, and 0.24 mg/100 g, respectively, and in group 5, 0.65, 0.44, and 0.26 mg/100 g, respectively.

Statistical treatment of the results showed that pigs with *hepatosis diaetetica* and the animals in group 5 which had received a diet deficient in vitamin E had significantly lower tocopherol levels in the heart, liver, and spleen than the pigs in group 2 which represent the normal levels for autopsy material.

The tocopherol levels in the heart and spleen of pigs with waxy muscle degeneration may possibly be significantly lower than the normal levels in group 2.

In order to study more closely the relationships between the tocopherol levels in different organs, an analysis of covariance was made using the tocopherol values for heart and liver as one variant pair and the values for heart and spleen as another. If, in a coordinate system, the *x*-axis repre-

sents the tocopherol level for heart and the y -axis the tocopherol level for liver and spleen, respectively, the regression lines for the averages of the groups will pass through zero and they are significantly straight lines. This indicates that the relationship between the tocopherol levels in heart, liver, and spleen are linear. From the angle of the regression lines for the average values of the groups, it is possible to state the general relationship between the tocopherol content of heart, liver, and spleen to be about 8: 5: 3. This relationship applies to all groups, fresh normal material, autopsy material in which the tocopherol level may sink after death, and material from animals with vitamin E deficiency.

1. Obel, A.-L. *Acta Pathol. Microbiol. Scand. Suppl.* **94** (1953).
2. Wanntorp, H. and Nordlund, G. *Arkiv Kemi Mineral. Geol.* **25 A** (1947) No. 6.

Received April 27, 1957.

Metal Ammine Formation in Solution

XI. Stability of Ethylenediamine Complexes and the Coordination Number of Chromium(II)

R. L. PECSOK* and JANNIK BJERRUM

Chemistry Department A, Technical University of Denmark, Copenhagen, Denmark

The coordination compounds of chromium(II) have been studied for many years; however, no stability constants have been reported for any of its complexes, nor has any experimental evidence been given of its coordination number. The difficulty of preparing chromium(II) solutions free from other metals (or acetate), the instability of chromium(II) toward oxidation, and the strong tendency of chromium(II) to form insoluble basic salts are serious obstacles to such an investigation. From the ligand field theory it is postulated¹ that chromium(II) complexes should show simi-

larity with copper(II) complexes, *i. e.* have a tetragonal bipyramidal configuration and the metal ion a characteristic coordination number of four. This has been verified by establishing the formation curve for the chromium(II) ethylenediamine complexes. Studies with other amines are in progress and will be reported elsewhere.

Solutions containing *ca.* 0.04 M chromous chloride, 0.35 M hydrochloric acid, and 1 M potassium chloride were titrated with concentrated solutions of ethylenediamine (*ca.* 5 M, and 1 M KCl), the hydrogen ion concentration being determined with the glass electrode. It was shown spectrophotometrically that below 6 M chloride ion, chloro complexes are insignificant. The formation curves giving the average ligand number as function of the exponent of the free ethylenediamine concentration were calculated according to Bjerrum². Three independent titrations are represented in Fig. 1. The fulldrawn curve is the theoretical formation curve calculated from the stability constants obtained. With a concentration of free amine higher than *ca.* 0.1 M, there is a considerable scatter in the experimental points because of the high initial acid concentration required to prevent precipitation of hydroxy compounds during the course of the titration. However, it is evident that the formation function of Cr(II)-en levels off at $\bar{n} = 2$. Preliminary spectrophotometric measurements show qualitatively that the bis(ethylenediamine) chromous ion has only a very slight tendency to take up more ethylenediamine. Further it is interesting to see that there is a distinct "pentammine" effect in the spectra just as in the corresponding cupric systems^{3,4} (see Table 1).

Table 1. Data for the absorption maximum of the aquo chromous ion and two chromium(II) ethylenediamine solutions.

$C_{Cr(II)}$ mole/l	C_{en} mole/l	λ_{max} $m\mu$	$1/\lambda_{max}$ cm^{-1}	ϵ_{max}
0.212	0	714	14 000	4.7
0.0375	0.57	547	18 300	25.1
0.0351	10.8	571	17 500	17.1

The consecutive formation constants were calculated in the usual way^{2,5}, and found to be $\log K_1 = 5.145$, $\log K_2 = 4.035$ at 25°. In Table 2 these values are compared with the earlier determined constants for the other divalent transition group

* Guggenheim Fellow 1956—57, on leave from the University of California, Los Angeles.