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Effects of Acute Dietary Zinc Deficiency in the Rat.*

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By means of a diet furnishing about 7 μg of Zn per rat daily, studies at the University of Wisconsin[1-4] have convincingly demonstrated the indispensability of Zn in the nutrition of rats, although the Zn-deficient diet permitted the experimental rats to gain about 8 g per rat weekly during the 10 week period on the diet.[4] The findings have invalidated the conclusion from our laboratory[5] several years ago that the element probably is not a dietary essential. We have recently prepared a diet furnishing not more than 2 to 4 μg of Zn per rat daily. Young rats restricted to it quickly developed extreme degrees of deficiency. Because the studies have been temporarily interrupted, owing to the transfer of one of us (H.G.D.) to another laboratory, we have decided to make a preliminary report on the work at this time.

Diet. The diet was: casein hydrolysate (tryptic)† 15.00, egg white (cooked) 3.00, sucrose 66.29, salts 5.71, butter fat 10.00, and Oleum Percomorphum 50%, 2 drops per 100 g diet (approximately 2800 vitamin A units and 400 vitamin D units).

Each rat was given a daily supplement containing the equivalent of 6.4 g liver, 40 μg of thiamin and 1.0 mg of choline. The liver concentrate,‡ dissolved in 6 volumes of water, was centrifuged. The supernatant solution was transferred to a Pyrex glass separatory funnel and the pH was adjusted to about 6. The solution was extracted repeatedly with dithizone (diphenyl thiocarbazone) dissolved in CCl_4. When no more Zn could be removed Zn-free dilute HCl was added until the pH was about 4. The excess of dithizone was removed with redistilled CCl_4. Traces of CCl_4 were removed by

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2 Stirn, F. E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 347.

3 Hove, E., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1937, **119**, 768.

4 Hove, E., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1938, **124**, 750.

5 Newell, J. M., and McCollum, E. V., *J. Nutrition*, 1933, **6**, 289.

† Obtained from Mead Johnson & Co., Evansville, Indiana, through the courtesy of Dr. Warren M. Cox.

‡ Obtained from the Lederle Laboratories, Pearl River, N. Y., through the courtesy of Dr. Guy W. Clark.

vacuum. The thiamin and choline were then added to the purified liver solution.

To purify the casein hydrolysate 225 g of the dry powder were stirred into 1700 ml of water. After saturation had occurred the mixture was centrifuged. The supernatant solution was adjusted to pH 6-7 with redistilled NH_4OH and extracted with dithizone as in the case of the liver preparation. The purified solution was evaporated to dryness *in vacuo,* using a Pyrex all-glass still, and dried over Drierite.

Water was added to commercial sucrose to make a 50% solution. After adjusting the pH with NH_4OH, the solution was extracted with dithizone as in the case of liver. It was reduced to a viscous syrup by *in vacuo* distillation and the sugar was precipitated by the addition of redistilled ethyl alcohol. It was washed several times with alcohol and dried at 60° C.

Egg white was prepared by drying the heat-coagulated white of fresh eggs at 60° C.

Butter fat was melted and washed 4 or 5 times with equal volumes of hot redistilled water.

The salt mixture was composed of $Na_2CO_3.H_2O$ 9.5, $CaHPO_4.2H_2O$ 21.5, KCl 15.2, $MgSO_4.7H_2O$ 9.8, $Fe_2(SO_4)_3$ 0.95, $CuSO_4.5\ H_2O$ 0.10, and $MnSO_4.3H_2O$ 0.05. The $Na_2CO_3.H_2O$, KCl and $MgSO_4.7H_2O$ were purified by extracting the concentrated aqueous solutions of each with dithizone in CCl_4. The purified solutions were concentrated and the salts precipitated by the addition of alcohol. The $CaHPO_4.2H_2O$ was prepared by dithizone extraction of a neutralized $CaCl_2.6H_2O$ solution and combining it with a K_2HPO_4 solution similarly extracted. The other salts were purified by recrystallization of the C.P. products.

Experimental and Results. Fifteen 24-26-day-old rats from 3 different litters were placed on the diet and supplements. Seven (controls) were given 0.66 mg $ZnSO_4.7H_2O$ (0.15 mg Zn) in 0.2 ml of H_2O per rat daily. All the rats were kept on monel metal screens in Pyrex glass jars. The diet was given *ad libitum.* Water was redistilled in Pyrex glass. Two times each week all rats were given a small supplement of salts of I_2, Cd, Mo, As and F in aqueous solution.

The control rats developed normally and gave every evidence that the diet, when supplemented with Zn, was adequate. Two of the Zn-deficient rats died, one after 33 days on the diet and the other after 61 days. During 11 weeks on the diet the Zn-deficient rats gained an average of only 4 g per rat weekly and the controls averaged 16 g. Growth became greatly retarded in the Zn-deficient rats after the

first 2 or 3 weeks. There was marked eczema in 2 cases. Some alopecia occurred in a few of the rats. Alkaline phosphatase[6] in the blood serum was markedly reduced but it was not affected in bone and kidney. The concentration of bone ash was decreased. In the experimental rats the average value was 55.8% and in the controls it was 62.2. There appeared to be some hemoconcentration. Carbonic anhydrase[7,8] of blood was slightly raised, but the enzyme activity per unit of red cells was unchanged. This is of special interest in relation to the finding that the purified enzyme contains 0.32% Zn.[9] The average concentration of Zn in the femurs of Zn-deficient rats was 94.7 μg per g of ash and in the controls it was 236.6 μg per g of ash. The method of Caughey, Holland and Ritchie[10] was used to estimate Zn. Detailed histological studies of the tissues will be published subsequently.

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Influence of H Ion Concentration on Depressant Effect of Nicotine Solutions on Ciliated Epithelium.

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Ellisor and Richardson[1] report a considerable difference between the toxicity of nicotine sulfate and the free alkaloid for gold fish. While they did not actually measure the ratio between these toxicities, they were able to derive it from calculations based on their own data, and found the base to be about 6 times more toxic than the salt. They also observed that the base penetrates into gold fish from 5 to 7.5 times more rapidly than the salt. In view of these observations it seemed desirable to ascertain whether or not a similar difference is to be observed in the action of nicotine and its salt on a relatively simple physiological system such as the ciliated epithelium. This tissue was selected because it may be obtained readily from the esophagus of

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7 Roughton, F. J. W., *Physiol. Rev.*, 1935, **15**, 241.

8 Lambie, C. G., *Edinburgh Med. J.*, 1938, **45**, 373.

9 Keilin, D., and Mann, T., *Nature*, 1939, **144**, 442.

10 Caughey, R. A., Holland, E. B., and Ritchie, W. S., *J. Assn. Off. Agric. Chem.*, 1938, **21**, 204.

1 Ellisor, L. O., and Richardson, C. H., *J. Cell. and Comp. Physiol.*, 1938, **11**, 377.