THE SODIUM CHLORIDE LEVELS AND THE EFFECT OF SODIUM CHLORIDE ADMINISTRATION ON THE ABNORMAL MANIFESTATIONS ASSOCIATED WITH A DEFICIENCY OF THE FILTRATE FACTORS OF VITAMIN B IN RATS*

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It has been reported and confirmed (1–3) that rats fed a diet deficient in the filtrate factors of vitamin B developed graying of the fur. Furthermore, under the same experimental conditions, certain pathological changes occurred in the adrenal cortex (4). The changes have consisted for the most part of hemorrhage, necrosis, and atrophy.

There has been ample evidence that the adrenal cortex was concerned with the metabolism of sodium and chloride (5, 6). In view of this, the question arose as to whether the manifestations associated with a deficiency of the filtrate factor in rats could be influenced by the amounts of sodium chloride in the diet. Furthermore, it seemed important to establish whether the changes produced in the adrenal cortex, as a result of the deficiency, were sufficient to affect the plasma level of chlorides.

Procedure

Rats of the Long-Evans strain were used for the studies. The animals were bred in the laboratory and litter mates were used. The rats were started on the experimental diet when 4 weeks old.

The basal diet consisted of casein 22 per cent, sucrose 64 per cent, primex 9 per cent, cod liver oil 2 cc. per 100 gm., and a modi-

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fied salt mixture 5 per cent (this contained ferric, potassium, and magnesium citrate, calcium diphosphate, copper sulfate, and potassium iodide). 12.5 cc. of Galen B were added to 100 gm. of the diet for the control rats. The diets of the experimental rats were supplemented with thiamine chloride, pyridoxine, and riboflavin, so that each rat received daily about 15 γ each of the first two and 45 γ of riboflavin.

Three animals were kept in a cage and they were allowed to eat the diet ad libitum. The diet contained no sodium chloride. The sodium chloride was given in water and placed in a drinking bottle with a curved glass tube outlet from which the rats drank directly, so that none was lost by leakage. The rats on the low salt intake were given a solution containing 0.064 per cent NaCl. Three rats consumed about 50 cc. of the salt solution daily. The salt solution selected for use in the diets with the low salt intake contained a concentration of NaCl equal to that found in Galen B. Since Galen B served as the source of the complete vitamin B complex in the diet of the control rats, the dilute salt solution used made the intake of the experimental and control rats comparable.

The rats on the high salt intake were given a 0.9 per cent solution of NaCl. Three rats were allowed 100 cc. daily of the solution.

Two groups of control rats were included in the study, one group of three rats on the high NaCl intake and one group of three rats on the low NaCl intake. As a complete source of the vitamin B complex 125 cc. of Galen B per kilo were added to the basal diet of the control animals.

At the end of the experimental period, when graying of the fur had occurred, blood was withdrawn from the tail. The tail was heated over an electric light bulb for a few moments, a piece was clipped off, and the tail was immediately inserted under oil into a small centrifuge tube. For the determination of the serum chloride Hald's modification of Paterson's micromethod was employed (7). In addition the serum protein was determined by the falling drop method (8).

When the animals were sacrificed, both adrenals were removed and sectioned. The results of the pathological studies, including observations on other organs, will be reported in detail in a separate communication. The adrenal findings are summarized briefly in
Tables I and II. We are indebted to Dr. Irving Graef of the Department of Pathology for a report of the pathological findings.

Results

In all, twenty-one rats were studied. Series C-1 consisted of nine rats, six of which were given the deficient diet plus the high salt intake. Three rats served as controls for this group and were given the experimental diet plus Galen B and the high salt intake. Series C-2 consisted of twelve rats. Nine rats were fed the deficient diet and the low salt intake. Three rats, serving as controls, were given the experimental diet plus Galen B. The only salt was that contained in the Galen B. The observations on all the animals were continued for an average period of 100 days.

The three control rats in both groups gained an average of 111 gm. during the period of observation. Their coats remained in excellent condition and there was no evidence of any graying. They were sacrificed at 99 and 110 days.

Series C-2 on Low Salt Intake (Table I)—The rats receiving the low salt intake failed to gain weight normally. At the end of the experimental period, the average weight was 83 gm. as compared with an average weight of 150 gm. in the three normal controls. Distinct and marked graying of the fur occurred in all but one animal in the experimental group. One animal died on the 53rd day of the experiment and in this rat the graying was obscured owing to a profound loss of fur. In the other rats the graying usually began over the head and back of the neck, spread uniformly down the sides of the body, and finally involved the entire body. In estimating the time of onset of the graying, we have reported the time when it was first observed and the time when graying had become pronounced. When first observed, a small patch of slightly gray fur was found over the head or back of the neck. The change in the color of the fur occurred about 10 days before graying became pronounced, by which time it had also spread over more of the body. As the experiment proceeded the fur over most of the body became silvery gray. In five of the nine rats the graying was severe. In three animals it was somewhat less intense and as previously mentioned in the rat which died on the 53rd day it was obscured, owing to the alopecia. The average time of onset of pronounced graying in the rats on the low salt diet was 43 days.
Relation of NaCl and Vitamin B

In one animal the graying was definite after 29 days and in one animal it was not distinct until the 62nd day. First graying of the fur, however, was observed in the rats on the low salt intake after an average period of 32 days. The range of the time of onset was from 29 to 47 days.

**Table I**

*Summary of Findings of Rats on Low Salt Intake. Series C-2*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Terminal serum Cl.</th>
<th>Terminal serum protein</th>
<th>Onset of graying</th>
<th>Definite day</th>
<th>Day killed</th>
<th>Failure to gain weight</th>
<th>Degree of graying</th>
<th>Alopecia</th>
<th>Dermatitis</th>
<th>Superficial hemorrhages of skin</th>
<th>Lipid depletion</th>
<th>Atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m.eq. per l.</td>
<td>gm. per cent</td>
<td>1st noted day</td>
<td>Definite day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtrate factor-deficient</td>
<td>39</td>
<td>105</td>
<td>5.95</td>
<td>29</td>
<td>39</td>
<td>83</td>
<td>S.</td>
<td>S.</td>
<td>M.</td>
<td>0</td>
<td>M.</td>
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<tr>
<td></td>
<td>40</td>
<td>109</td>
<td>5.95</td>
<td>29</td>
<td>39</td>
<td>83</td>
<td>&quot;</td>
<td>S.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>S.</td>
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<tr>
<td></td>
<td>41</td>
<td>108</td>
<td>4.66</td>
<td>29</td>
<td>29</td>
<td>96</td>
<td>M.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0</td>
<td>Sl.</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>(91)†</td>
<td>(6.15)†</td>
<td>39</td>
<td>46</td>
<td>100</td>
<td>Sl.</td>
<td>M.</td>
<td>Sl.</td>
<td>0</td>
<td>M.</td>
<td>&quot;</td>
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<tr>
<td></td>
<td>43</td>
<td>106</td>
<td>5.14</td>
<td>29</td>
<td>39</td>
<td>98</td>
<td>M.</td>
<td>&quot;</td>
<td>S.</td>
<td>S.</td>
<td>S.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>96</td>
<td>5.17</td>
<td>39</td>
<td>47</td>
<td>99</td>
<td>&quot;</td>
<td>S.</td>
<td>0</td>
<td>M.</td>
<td>M.</td>
<td>Ma.</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>101</td>
<td>5.81</td>
<td>39</td>
<td>39</td>
<td>110</td>
<td>&quot;</td>
<td>M.</td>
<td>M.</td>
<td>0</td>
<td>S.</td>
<td>Sl.</td>
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<tr>
<td></td>
<td>46</td>
<td>100</td>
<td>5.48</td>
<td>29</td>
<td>39</td>
<td>110</td>
<td>&quot;</td>
<td>S.</td>
<td>&quot;</td>
<td>0</td>
<td>M.</td>
<td>0</td>
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<tr>
<td>Control</td>
<td>47</td>
<td>98</td>
<td>6.19</td>
<td>47</td>
<td>62</td>
<td>110</td>
<td>S</td>
<td>&quot;</td>
<td>S.</td>
<td>0</td>
<td>S.</td>
<td>Sl.</td>
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<tr>
<td></td>
<td>48</td>
<td>99</td>
<td>5.99</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>100</td>
<td>5.66</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>50</td>
<td>98</td>
<td>5.92</td>
<td>0</td>
<td>0</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Sl.</td>
</tr>
</tbody>
</table>

* The adrenal pathology was limited to the juxtamedullary zone.
† Blood hemolyzed.

In addition, five of the animals on the low salt intake developed a severe alopecia. The fur was brittle and patches of it fell out, exposing areas of skin. In four rats there was a generalized dermatitis and in seven of the animals there were superficial hemorrhages over the feet.

The adrenal changes were confined to the juxtamedullary zone of the cortex. There was no evidence of hemorrhages in any of the
adrenals. The adrenals in seven of the animals showed from slight to marked lipid depletion. In only two of the adrenals was any atrophy of the cortical cells found.

The general appearance of the animals was poor, as is shown in Fig. 1. The rats on the low salt intake appeared to be in a poorer state of nutrition than the experimental rats on the high salt intake.

**Series C-1 on High Salt Intake (Table II)**—The experimental rats on the high salt intake also failed to gain weight, but the failure was not as pronounced as in the rats on the low salt diet. Interestingly enough, only three of the six rats developed graying of the fur and in one rat it was always slight. The graying that did occur was first observed on the 75th day and did not become pronounced until about the 100th day. The onset of definite graying in the rats on the high salt intake was 55 days later than the onset in the rats on the low salt intake.

Alopecia was present to a slight or moderate degree in four of the rats on the high salt intake and four of the animals also showed some superficial hemorrhages over the feet. On pathological examination the adrenals of four rats showed a slight amount of lipid depletion and in one rat the depletion was moderate. Severe
110  Relation of NaCl and Vitamin B

atrophy of the cortical cells occurred in two others. A moderate amount of atrophy was observed in the adrenals of two of the control rats. The adrenal changes were again confined to the juxtamedullary zone of the cortex.

*Serum Chloride and Protein Values*—The serum chloride values in the rats receiving the low salt intake were within normal limits. In milliequivalents per liter, the serum chloride varied from 96 to

**Table II**

Summary of Findings of Rats on High Salt Intake. Series C-I

0 = none, S. = severe, M. = moderate, Sl. = slight.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Terminal serum NaCl mg. per l.</th>
<th>Terminal serum protein gm. per cent</th>
<th>Onset of graying</th>
<th>Day killed</th>
<th>Degree of graying</th>
<th>Alopecia</th>
<th>Surface hemorrhages of skin</th>
<th>Adrenal changes*</th>
<th>Lipid leucocytosis</th>
<th>Atrophy</th>
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<tr>
<td>51</td>
<td>7.14</td>
<td>75</td>
<td>100</td>
<td>110</td>
<td>M.</td>
<td>Sl.</td>
<td></td>
<td></td>
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<tr>
<td>52</td>
<td>6.66</td>
<td>100</td>
<td>?</td>
<td>110</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td></td>
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<tr>
<td>53</td>
<td>7.04</td>
<td>75</td>
<td>100</td>
<td>110</td>
<td>Sl.</td>
<td>S.</td>
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<td>54</td>
<td>4.56</td>
<td>0</td>
<td>0</td>
<td>96</td>
<td>M.</td>
<td>0</td>
<td></td>
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<td></td>
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<tr>
<td>55</td>
<td>0</td>
<td>96</td>
<td>0</td>
<td>0</td>
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<td>Sl.</td>
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<td>56</td>
<td>5.44</td>
<td>0</td>
<td>0</td>
<td>99</td>
<td>Sl.</td>
<td>0</td>
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<tr>
<td>57</td>
<td>5.66</td>
<td>0</td>
<td>0</td>
<td>96</td>
<td>0</td>
<td>Sl.</td>
<td></td>
<td>M.</td>
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<tr>
<td>58</td>
<td>6.94</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>M.</td>
<td>Sl.</td>
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<tr>
<td>59</td>
<td>6.02</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
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<td>Sl.</td>
</tr>
</tbody>
</table>

*The adrenal pathology was limited to the juxtamedullary zone.

109. The serum protein varied from 4.66 to 6.19 gm. per cent in this group. In the three control rats, kept on a low salt intake, the serum chloride levels were 98, 99, and 100 milliequivalents per liter and the serum protein was quite uniform and averaged 5.86 gm. per cent. In the experimental rats on the high salt intake the terminal serum chloride values ranged from 102 to 107 milliequivalents per liter. The serum protein values varied from 4.6 to 7.1 gm. per cent. The serum chloride levels in the control animals in this group ranged from 97 to 108 milliequivalents per liter and the serum protein values ranged from 6.9 to 5.7 gm. per cent.
DISCUSSION

Of the fifteen rats observed on diets deficient in the filtrate factors of the vitamin B complex, those in which the salt intake was kept very low developed pronounced graying of the fur. Furthermore the graying occurred much sooner and the incidence was greater in the rats on the low salt intake than in the rats on the high salt intake. In addition the rats on the low salt ration developed the other skin manifestations associated with this deficiency (2, 9), such as alopecia, dermatitis, and superficial hemorrhages of the skin. It seems clear that the amount of salt added to the filtrate factor-deficient diet influenced the incidence, extent, and degree of graying of the fur that occurred in the rats. Morgan (10) mentioned that “three dogs were placed on a salt-free . . . diet, and this complicated the effect of the vitamin deficiency in an unexpected way.” No further reference was made to the animals in this report, so that it was not clear what the complications were.

The adrenal changes that were observed in the experimental animals on the low salt intake were mostly those of lipid depletion of the juxtamedullary zone of the cortex. In the experimental animals on the high salt ration, lipid depletion of the cortex also occurred and in addition a moderate to severe degree of atrophy was present in the adrenals of four rats.

Daft and Sebrell (4) have reported hemorrhage, necrosis, and atrophy of the adrenal cortex in rats kept on a diet deficient in the filtrate factors. Nicotinic acid and choline were added to the diets used in their experiments. The fact that neither hemorrhage nor necrosis was observed in the adrenals of the rats we have studied raises the question as to whether the more severe changes reported by other investigators may not have been due to the toxic effects of nicotinic acid or choline in the absence of the filtrate factors. The amount of salt added to the diet did not seem to protect the adrenal cortex from atrophy or lipid depletion.

In none of the rats was the extent of the adrenal pathology severe enough to influence the serum chloride values. We confined ourselves to the chloride determinations in these experiments, because the micromethod for sodium determinations is not entirely satisfactory. The sodium level in the plasma is also definitely influenced by the adrenal cortex hormone, but any significant change in the serum sodium is usually associated with changes in the
Relation of NaCl and Vitamin B

cloride level. It might well be that more pronounced pathological damage of the adrenal cortex would influence the level of chloride in the blood and experiments are in progress which we hope will answer this question. The fact that the terminal serum proteins were normal showed that no profound hemoconcentration had occurred in these animals.

SUMMARY

Fifteen rats of the Long-Evans strain were placed on diets deficient in the filtrate factor of the vitamin B complex. In nine of these animals the NaCl content of the diet was kept at a very low level. The other six animals were given large amounts of NaCl. Six control rats were studied on diets adequate in the entire vitamin B complex, three on a low salt intake and three on a high salt intake.

The rats on the low salt intake developed graying of the fur, on an average, 55 days sooner than did the animals on the high salt intake. Furthermore the graying was more pronounced and occurred in all but one of the nine rats.

Atrophy of the cells in the juxtamedullary zone of the adrenal cortex occurred to a greater extent in the animals on the high salt intake. The lipid depletion was more profound in the adrenal cortex of the rats on the low salt intake. In none of the animals were hemorrhages of the adrenal cortex observed.

The serum chloride levels, determined in all of the animals at the end of the experimental period, were within normal limits, as were the serum protein values. Obviously the pathological changes that did occur in the adrenal cortex of the rats were not sufficient to influence the chloride levels of the serum. The fact that the serum protein values were within normal limits indicated that hemoconcentration did not occur.

BIBLIOGRAPHY

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