On the Etiology of “Meat Anemia” in Mice

By Judith Ilan, M. Kende and K. Guggenheim

It was recently reported by Adler1 that mice kept on a diet of meat, boiled or fresh, eventually develop an anemia which is generally fatal. The present paper describes experiments which were designed with the aim of elucidating the factors involved in the etiology of this anemia.

The general plan of our study was as follows: (1) to compare weight, erythrocyte counts and hemoglobin levels of mice kept for 6 weeks on a diet composed of meat exclusively, with corresponding values in control mice; (2) to study the effect of certain kinds of treatment in mice subsisting on the meat diet. These preventive trials also lasted 6 weeks; (3) to examine the effect of certain therapeutic methods on mice rendered anemic by the meat diet. These therapeutic trials lasted 3 weeks.

Methods

Young Swiss mice, 3 weeks old, were used. They were kept on one of the following three diets: (1) meat diet, consisting of raw beef muscle; (2) a semisynthetic diet composed of casein (18 per cent), cornstarch (36 per cent), glucose (37 per cent), vegetable oil (5 per cent), salts (U.S.P. XIV; no. 2; 4 per cent) and the conventional vitamins; (3) stock diet; this diet contains 20 per cent protein and consists of sprouted wheat, barley, bran, skim milk powder and an occasional addition of cod liver oil.

Vitamin B12 levels were determined with an E. coli mutant, as described by Aronovitch and Grossowicz.2 Hemoglobin was examined in tail blood by the cyanmethemoglobin method. Red blood cells were counted in the usual way, using tail blood.

The liver extract used was prepared by the manufacturer (Teva, Middle East Pharmaceutical and Chemical Works, Jerusalem) as follows: Dried liver powder was digested with papain, extracted with alcohol and filtered. The filtrate was concentrated by vacuum distillation until it contained 280 mg. of dry substance per milliliter. It was found to contain 30 μg. vitamin B12 per milliliter.

Results

One group of mice was fed the meat diet, while controls received the stock and semisynthetic diets, respectively. Table 1 (experiments 1, 2 and 3) and figure 1 show the effects of these diets on weight, erythrocyte counts and hemoglobin levels. Twenty-one of 50 mice on the meat diet succumbed during the 6 weeks’ observation period, whereas all of the 32 control mice remained alive. Weight of the experimental mice increased by 4 Gm. only, as against 14 and 15 Gm., respectively, in the control groups. This is partly due to a lower caloric intake of the experimental mice, which consumed 9

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Table 1.—Effects of Various Treatments on Mortality, Weight, Erythrocyte Count and Hemoglobin in Mice Kept on a Meat Diet for 6 Weeks (Means and standard errors)

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>No. of mice at start of exp.</th>
<th>Diet</th>
<th>Treatment</th>
<th>Mortality in 6 weeks</th>
<th>Initial*</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weight (Gm.)</td>
<td>Erythrocytes (10^6/cu.mm.)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>Stock</td>
<td>——</td>
<td>0/10</td>
<td>10.3 ± 0.24</td>
<td>7.20 ± 0.22</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>Semi-synthet.</td>
<td>——</td>
<td>0/22</td>
<td>11.3 ± 0.30</td>
<td>6.49 ± 0.15</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Meat</td>
<td>——</td>
<td>21/50</td>
<td>12.3 ± 0.40</td>
<td>6.70 ± 0.20</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td></td>
<td>5 µg. PABA, subc.</td>
<td>1/10</td>
<td>10.4 ± 0.26</td>
<td>6.62 ± 0.20</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td></td>
<td>5 µg. folic acid sube.</td>
<td>9/20</td>
<td>12.5 ± 0.70</td>
<td>6.28 ± 0.40</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td></td>
<td>0.01 µg. B12 sube.</td>
<td>4/10</td>
<td>10.3 ± 0.21</td>
<td>6.96 ± 0.09</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td></td>
<td>5 µg. PABA, 5 µg. folic acid and 0.01 µg. B12 sube.</td>
<td>9/20</td>
<td>12.4 ± 0.67</td>
<td>6.77 ± 0.28</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td></td>
<td>as 7 per os</td>
<td>1/10</td>
<td>12.3 ± 0.42</td>
<td>8.33 ± 0.20</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td></td>
<td>0.1 ml. liver extract, subc.</td>
<td>2/10</td>
<td>11.0 ± 0.69</td>
<td>5.96 ± 0.38</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td>10 µg. pyridoxine sube.</td>
<td>4/10</td>
<td>9.3 ± 0.33</td>
<td>7.22 ± 0.99</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td></td>
<td>1.5 mg. ferrous sulfate per os</td>
<td>6/10</td>
<td>10.1 ± 0.76</td>
<td>6.57 ± 0.89</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td></td>
<td>50 IU. vitamin A weekly per os</td>
<td>6/10</td>
<td>9.9 ± 0.42</td>
<td>6.90 ± 0.21</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td></td>
<td>Penicillin†</td>
<td>3/10</td>
<td>10.3 ± 0.60</td>
<td>5.82 ± 0.30</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td></td>
<td>Aureomycin†</td>
<td>3/10</td>
<td>9.9 ± 0.10</td>
<td>6.02 ± 0.25</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td></td>
<td>Dilution of meat with beef liver (1 part liver, 3 parts meat)</td>
<td>3/10</td>
<td>12.3 ± 0.50</td>
<td>6.05 ± 0.37</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td></td>
<td>Dilution of meat with casein mixture†</td>
<td>0/10</td>
<td>11.0 ± 0.40</td>
<td>5.84 ± 0.25</td>
</tr>
</tbody>
</table>

*Figures refer only to those mice which completed the experiment.
150 mg. antibiotic incorporated into 1 Kg. minced meat.
15.5 Gm. casein, 4.0 Gm. cornflour and 0.5 Gm. minerals (U.S.P. XIV) and 15.0 ml. water were mixed with 75 Gm. minced meat.
calories approximately per day, as against 18 calories approximately, the energy value of the daily food of the controls. There were only small changes in erythrocyte counts and hemoglobin levels in the two control groups and a marked decrease in the mice on the meat diet. Similar results were obtained in a therapeutic trial (table 2, experiments 17 and 18). Feeding of the semisynthetic diet to 6 anemic mice resulted in a considerable recovery of erythrocytes, hemoglobin and weight. The meat diet, however, caused a further decrease in all these values.

Next the question was studied, whether the deleterious effect of the meat diet is due to dietary lack or impaired availability of essential nutrients which may have a hematopoietic action. Since the typical course of infection with Plasmodium vinckei is suppressed in mice kept on the meat diet, and since treatment with para-aminobenzoic acid (PABA) abolishes this suppressive
effect, treatment with PABA was tried first. Ten mice were fed the meat diet and subcutaneously injected with 5 μg PABA per day. As table 1 (experiment 4) demonstrates, this treatment inhibits the anemia but has no effect on the growth depression. Since PABA forms a part of the molecule of folic acid, a group of 20 mice was treated with daily subcutaneous injections of 5 μg of this vitamin (experiment 5). These mice experienced a high mortality; their hemoglobin values were higher than those of untreated controls, whereas no effect was seen on erythrocytes and weight.

A similar result was obtained with vitamin B₁₂, which was injected in daily doses of 0.01 μg. (experiment 6). It may be noted that the meat anemia is actually accompanied by an increased storage of vitamin B₁₂ in the organs of the anemic mice, as follows from table 3. The experiment in this table comprises 5 groups of mice which were three weeks old. One group was sacrificed at the start of the experiment and the vitamin B₁₂ content of liver, kidneys, spleen, muscle and heart determined. Two groups received the semisynthetic diet and two groups the meat diet. One of each of these two groups was injected with 0.01 μg vitamin B₁₂ per day. The animals were sacrificed after six weeks and the vitamin B₁₂ contents of their organs examined. As can be seen, treatment with vitamin B₁₂ increases the B₁₂ content of the organs of mice on either diet. Moreover, mice on the meat diet which were not treated with B₁₂ had higher B₁₂ values than mice on the semisynthetic diet and treated with the vitamin. This result is understandable in view of the B₁₂ content of meat, which was found to be 0.03 μg/gm. Since the mice ate approximately 5 Gm. meat per day, they consumed about 0.15 μg B₁₂, which is much more than the dose injected. This experiment shows, therefore, that the meat anemia is not due to dietary lack or unavailability of vitamin B₁₂.

Subcutaneous injections of PABA, folic acid and vitamin B₁₂ (experiment 7) resulted in a somewhat improved weight increase and had a only small effect on hemoglobin level, whereas the mortality remained high and the erythrocyte count low. Peroral administration of these three substances was without effect on weight and erythrocytes, but significantly increased hemoglobin (experiment 8). Liver extract (experiment 9) did not prevent anemia but alleviated it.

Anemia has frequently been observed when rats, dogs, pigs or monkeys were kept on a pyridoxine-deficient diet. Although beef muscle is a rich source of this vitamin, the possibility of an impaired availability for mice in a diet composed of raw meat only cannot be excluded. Twelve mice were therefore injected daily with 10 μg pyridoxine while subsisting on the meat diet. This treatment had a distinct inhibiting effect on the decrease of hematologic values without affecting, however, the stunted growth and the high mortality. For similar reasons the effect of iron (1.5 mg ferrous sulfate, given per os, experiment 11) was studied; it had no general but a slight hematologic effect.

Since the meat diet is probably deficient in vitamin A, it was thought that lack of this vitamin might be a factor in the development of the anemia. Moreover, reduced values of erythrocytes and hemoglobin have been reported in vitamin A deficient rats. Peroral administration of doses as high
as 50 I.U. per week did not, however, prevent the anemia (experiment 12). These experiments show that administration of usual hematopoietic factors or of nutrients in which the meat diet may be deficient did not completely prevent the anemia, although some inhibiting effect was apparent.

It was, therefore, thought possible that changes of the intestinal flora resulting from this peculiar diet which may lead to a lack of some essential hematopoietic factor(s), synthesized by the normal flora, are responsible for the meat anemia. We therefore fed a meat-penicillin and a meat-aureomycin diet to two groups of animals (50 mg. antibiotic per kilogram of minced meat, experiments 13 and 14). Mice kept on the meat-penicillin diet had much higher hemoglobin values than those receiving meat without the antibiotic. The effect on erythrocytes was, however, very small and that on growth absent. With aureomycin no effect was noted. It is therefore possible that the intestinal flora is involved in the etiology of the meat anemia, although this possibility is not proved.

In a further search for possible hematopoietic factor(s) lacking in meat but essential for mice one part of four of the meat diet was replaced by beef liver (experiment 15). This replacement suppressed the anemia completely; it had, however, no effect on growth. Moreover, beef liver had a striking therapeutic effect in anemic mice (table 2, experiment 19). This effect of liver may, however, at least partially be due to the dilution of meat as evinced from experiment 16. In this experiment meat was diluted with a casein mixture which was composed of casein, cornstarch, minerals and water which was isocaloric and isonitrogenous with liver. Dilution of meat with this mixture has a similar anemia-preventing effect as liver but was inferior to it in restoring low erythrocyte and hemoglobin values in anemic mice (table 2, experiment 20).

**Discussion**

Our experiments demonstrate that young mice kept on a diet composed of meat only eventually develop an anemia which is accompanied by stunted growth and a high mortality. None of the well known hematopoietic factors, such as folic acid, vitamin B₁₂, liver extract or iron, or even a combination of some of them, are able to prevent decisively the hematologic deterioration, although some beneficial effect has been seen with some of these treatments. The meat anemia appears also not to be caused by lack of vitamin A, PABA or pyridoxine, in which the meat diet is deficient or which may be present in meat but not to be nutritionally available to mice.

Similarly, changes in the intestinal flora resulting from this peculiar diet, as far as they may be influenced by peroral administration of antibiotics, do not seem to be involved in the etiology of the anemia, although they cannot be excluded. We have not as yet investigated the changes in the intestinal flora. In normal mice we found the cecum packed with *Trichomonas muris*, but in the anemic animals these flagellates were scarce. The significance of this finding still remains to be determined. It is interesting to note that Hegner⁶ noted a decrease in the number of trichromonads in rats fed on a carnivorous diet.
Table 2.—Effects of Various Treatments on Mortality, Weight, Erythrocyte Count and Hemoglobin of Mice Rendered Anemic by a Meat Diet

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>No. of mice at start</th>
<th>Diet</th>
<th>Treatment</th>
<th>Mortality in 3 weeks</th>
<th>Weight (Gm.)</th>
<th>Erythrocytes (10⁹/cu.mm.)</th>
<th>Hemoglobin (Gm./100 mL)</th>
<th>Weight (Gm.)</th>
<th>Erythrocytes (10⁹/cu.mm.)</th>
<th>Hemoglobin (Gm./100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>10</td>
<td>Meat</td>
<td>——</td>
<td>7/10</td>
<td>14.6 ± 1.4</td>
<td>3.04 ± 0.13</td>
<td>6.6 ± 0.8</td>
<td>13.4 ± 1.7</td>
<td>2.84 ± 0.54</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>18</td>
<td>6</td>
<td>Semi-synthetic</td>
<td>——</td>
<td>0/6</td>
<td>15.3 ± 1.1</td>
<td>2.16 ± 0.24</td>
<td>4.9 ± 0.4</td>
<td>24.0 ± 1.1</td>
<td>6.47 ± 0.15</td>
<td>16.0 ± 0.5</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>Meat</td>
<td>Dilution of meat with beef liver (1 part liver, 3 parts meat)</td>
<td>2/6</td>
<td>17.0 ± 1.8</td>
<td>2.26 ± 0.10</td>
<td>5.8 ± 0.9</td>
<td>21.0 ± 0.9</td>
<td>6.82 ± 0.23</td>
<td>15.8 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>Meat</td>
<td>Dilution of meat with casein mixture†</td>
<td>9/14</td>
<td>20.0 ± 0.9</td>
<td>2.18 ± 0.20</td>
<td>4.7 ± 0.7</td>
<td>15.6 ± 1.4</td>
<td>2.38 ± 0.78</td>
<td>7.6 ± 1.6</td>
</tr>
</tbody>
</table>

Observation time, 3 weeks.

*Figures refer only to those mice which completed the observation period.

†See † footnote in table 1.
Dilution of the meat diet with well defined nutrients, i.e., casein, cornstarch and minerals, has, however, a strong inhibitory effect on the development of the anemia, although it is of no therapeutic value. Dilution of meat with liver prevented and cured the meat anemia. It appears, therefore, that the effect of this diet results not only from the dilution of meat but may also be due to the presence of some hematopoietic factor(s) lacking in mice which subsist on meat only.

Lack or impaired nutritional availability of well defined nutrients or hematopoietic factors can probably be excluded. On the other hand, the beneficial effect of dilution of meat with nutrients which appear to be hematopoietically inert may be interpreted by dilution of a hematopoiesis-inhibiting factor present in meat and/or by changes in the intestinal flora normally supplying the organism with factor(s) stimulating hematopoiesis. The existence of such factor(s), though not corroborated by our studies with antibiotics, can nevertheless not be excluded.

The meat diet appears to be nutritionally grossly inadequate, as seen from the depressed growth of mice subsisting on it. It may be interesting to note that the nutritional inadequacy of a meat diet for small rodents was already observed 100 years ago. Although replacement of one of four parts of meat by either beef liver or an isocaloric-isonitrogenous casein-glucose mixture prevents anemia, this dietary manipulation is not accompanied by a considerable improvement of growth. Thus, the development of the anemia is not mediated by a general deterioration of the state of nutrition. Depressed growth may be present in mice with almost normal hematologic values (table 1, experiments 15, 16).

In some series a lack of parallelism in decrease of red blood cells and of hemoglobin was found (e.g., experiments 5, 12, 13), which is explained by the relative inaccuracy of red blood cell counts. We lay more stress on the hemoglobin values than on erythrocyte numbers. Further studies aimed at a characterization of the meat anemia and using heart blood and bone marrow smears are now in progress. For the present we would like to mention that in the final stages the red cells show anisocytosis and abnormal cells, mainly macrocytes, and fewer microcytes, and that the bone marrow is megaloblastic.

**SUMMARY**

Young mice kept on a diet consisting of meat only develop an anemia which is accompanied by depressed growth and high fatality.
Treatment of mice while subsisting on the meat diet with para-aminobenzoic acid, folic acid, vitamin B₁₂, liver extract, pyridoxine, iron, vitamin A, penicillin, aureomycin or a combination of some of these substances neither prevents the anemia nor the mortality, nor does it improve growth. Some of these substances have, however, a slight effect in preventing anemia.

Replacing one of four parts of meat by beef liver or an isocaloric-isonitrogenous mixture of casein, cornstarch, salts and water almost completely prevents the anemia but does not improve, or only slightly improves, the stunted growth. Dilution of meat with liver but not with the casein mixture has a striking therapeutic effect in mice rendered anemic by the meat diet.

It is assumed that the anemia is caused by the presence in meat of a hematopoiesis-inhibiting factor and/or by the lack of some unknown hematopoietic factor(s) possibly produced by the intestinal flora and present in liver.

SUMMARIO IN INTERLINGUA
Juvene muses que es mantenite con un dieta consistente exclusivamente de carne disveloppa un anemia associate con depression del crescentia e con alte mortalitate.

Le tractamento del muses, durante que lor dieta es restringite a carne, con acido para-aminobenzoic, acido folic, vitamina B₁₂, extracto de hepate, pyridoxina, ferro, vitamina A, penicillina, aureomycina, o un combination de plures de iste agentes preveni ni le anemia ni le mortalitate e non meliora le crescentia. Certes del substantias listate reduce le grado del anemia leveemente.

Le substitution, pro un quarto del carne, de hepate bovin o de un mixtura isocaloric e isonitrogenic de caseina, amylo de mais, sal, e aqua preveni le anemia quasi completamente sed meliora non del toto o solo leveemente le retardo del crescentia. Le dilution del carne con hepate exerce un frappante effecto therapeutic in muses anemic per le dieta de carne. Le dilution del carne con le mixtura a caseina non ha iste effecto.

Es suponite que le anemia es causate per le presentia in le carne de un factor que inhibi le hematopoiese c/o per le absentia del un o del altere incognoscite factor hematopoietic que es possibilemente producite per le flora intestinal e que es presente in le hepate.

REFERENCES
On the Etiology of "Meat Anemia" in Mice

JUDITH ILAN, M. KENDE and K. GUGGENHEIM