THE EFFECTS OF VITAMIN DEFICIENT DIETS ON RATS, WITH SPECIAL REFERENCE TO THE MOTOR FUNCTIONS OF THE INTESTINAL TRACT IN VIVO AND IN VITRO.

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(A) Introduction.

In 1914 Keith (1) described the macroscopic and microscopic lesions which he found in a number of large intestines removed at operation for conditions of advanced colonic stasis. In 1921 I had the privilege of examining this material and undertook the experimental study which arose out of the anatomical findings. The salient features of these lesions were:—

(a) Accumulation of large cells with indistinct nuclei and numerous brown granules in their cytoplasm in the stroma under the epithelium and above the muscularis mucosae. They are sharply localised to that area, often bathed in lakes of lymph and strictly limited to the colon.

(b) Distinct pathological lesions often found in Auerbach's plexus, ranging from inflammatory cell infiltration and necrosis to extreme fibrosis.

(c) The epithelium is often flattened toward the lumen and sometimes so laden with brown pigment that the nucleus is seen with difficulty. The latter is often vesicular and appears unstained. Here and there the goblet cells are seen to form bladders and occasionally appear to burst into the reticularis evidently because a tough film which covers their surface prevents their proper discharge into the lumen.

(d) Occasional fibrosis of the muscular coats and thickening of the submucous connective tissue.

The accumulation of the pigment-bearing cells in the stroma and the pathological condition of Auerbach's tissue appeared to me very suggestive. Without going into a lengthy discussion on the neurogenic and myogenic theories of intestinal activity, the fact that this is still an unsettled point makes the discovery of the nervous lesion of particular interest. Clark and I (2) have recently furnished additional
evidence that Auerbach's plexus is of prime importance in maintaining the tone of the intestine and in initiating and carrying out peristalsis which is really a complex wave of altered intestinal tone. In 1919 McCarrison (3) published a report on the intestinal lesions of animals fed on certain vitamin deficient and otherwise unbalanced diets. Among the most marked changes, which included congestive, necrotic, and inflammatory changes in the mucous membrane and atrophy of the myenteron, he found also degeneration in Auerbach's plexus. He drew attention to these findings and suggested that vitamin deficient and ill-balanced diets which can produce such lesions may be a possible source of intestinal stasis in the human.

If degenerative changes of Auerbach's plexus could be produced in such a way, it seemed to me that here was a method of investigation which should yield interesting results both on the physiology of the intestinal activity as well as on the pathology of constipation, for whether or not the pathological conditions in the colons from cases of obstinate constipation which Keith had described resulted from vitamin deficiencies, the occurrence in both of a characteristic and unusual lesion seemed well worth following up.

My experiments were designed to determine whether pure vitamin deficiencies produce an alteration in intestinal activity in rats and also to determine the general anatomical changes resulting from these deficiencies. The latter investigation was necessary because the descriptions of the pathological changes resulting from vitamin deficiencies which appear in the literature are based for the most part on tissues from dead or dying animals, and represent extreme conditions often complicated by inanition. Furthermore, the diets largely used have been grossly unbalanced, so that the results in any case cannot be held to be those following pure vitamin deficiencies. My material is from animals which were not moribund and which were fed on vitamin deficient diets otherwise well balanced.

Before proceeding to the discussion of my experimental results it will be essential to give a detailed description of my observations on so-called "normal" control animals. It seems to me that sufficient emphasis has not been laid on this point. In most of the literature dealing with functional and particularly anatomical investigations on vitamin deficiencies, it is more or less assumed that normal control animals are in the greater proportion of cases normal, and little attention is paid to the wide individual variations found especially on microscopic examination. As will, however, be seen in the description which follows, laboratory animals, particularly albino rats, show extraordinarily high individual variations and morbidity. It is only after this is fully studied and analysed in a sufficiently large number of cases that the results following experimental diets assume their true significance, and this is usually strikingly different from the conclusions which would be drawn without such a preliminary study on controls.
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(B) Technique employed in these studies.

In all I used 217 rats. The general plan was to divide the rats into groups of 6 to 48, placing three in a cage. The sexes were divided and an approximately equal number of bucks and does was used in each experiment. At various intervals the rats were killed for isolated intestine tracings, histological material, and adrenalin estimations.* Because hyperadrenalinæmia was strongly suggested as responsible for many of the signs and symptoms of vitamin B deficiency(4), some of the groups received daily intraperitoneal injections of adrenalin in an attempt to reproduce the lesions said to be characteristic of this deficiency. The dose injected was 1/10 c.c. of 1/2000 solution of adrenalin chloride (Parke Davis) per 100 grms. of rat. Other animals were unilaterally adrenalectomised. It was suggested that if any diminution in intestinal activity were found this might be accounted for on a basis of general muscular atrophy involving the intestinal musculature. Several groups of rats were accordingly given daily intraperitoneal injections of pituitary extract in the hope that this would tend to cause hypertrophy of the bowel. The dose injected was 1/10 c.c. Parke Davis pituitrin per 100 grms. of rat.

During the course of the feeding, notes were taken on the general behaviour of the animals, the temperatures and weights were determined twice a week under as similar conditions as possible, and the weight of food intake and character of faeces were noted.

The diets which I employed were well balanced and were made up according to the formulae shown in table I. Hereafter in this paper, diet deficient in vitamin A will be referred to as A diet, that deficient in B as B diet, and in C as C diet. P diet contains in adequate quantity all the known vitamins.

Table I.

Table of the diets used, showing the proportions of the ingredients in parts by weight.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Casein 20 parts.</th>
<th>Starch 50 parts.</th>
<th>Hardened fat or butter 10 parts.</th>
<th>Salt mixture 8 parts.</th>
<th>Marmite 5 parts.</th>
<th>Lemon juice 5 parts.</th>
<th>Water 30 parts.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>casein</td>
<td>starch</td>
<td>butter</td>
<td>salt mixture</td>
<td>marmite</td>
<td>lemon juice</td>
<td>water</td>
</tr>
<tr>
<td>A</td>
<td>heated</td>
<td>..</td>
<td>hardened fat</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>B</td>
<td>extracted</td>
<td>..</td>
<td>butter</td>
<td>..</td>
<td>none</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>C</td>
<td>casein</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>marmite</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>

The butter was mixed with 2 per cent. of its bulk of cod liver oil. The heated casein was prepared by heating shallow layers of casein in a dry oven for 24 hours at a temperature of 110° Centigrade. The extracted casein was prepared by extracting

* The results of the adrenalin estimations are discussed in Biochemical Journal, 1923, xvii., 569.
casein with boiling 95 per cent. alcohol for 4 hours. "Marmite" is a commercial preparation of autolysed yeast. The hardened fat used was hydrogenated cotton-seed oil. The salt mixture was as follows, in parts by weight:—

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>5.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>7.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium acid phosphate</td>
<td>10.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>28.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.90</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.54</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.20</td>
</tr>
</tbody>
</table>

Except where the brain was wanted for examination, the rat was killed by a blow on the head with a metal rod. A fore limb was immediately amputated as close to the trunk as possible and the animal bled into a clean glass receptacle. The blood could thus be used for various tests.

As general fixative for tissues I used a 10 per cent. solution of neutralised formalin in physiological saline. This fixative has many shortcomings, but in view of the large amount of varied material that it was necessary to handle, it was chosen for its expediency. For special purposes osmic vapour was used according to the method of Cramer (5).

(O) Observations on 48 "normal" control rats.

The rats were purchased from dealers and weighed 125 grms. and over. It would have been much more advisable to have bred my own rats, but unfortunately neither time nor facility made this possible. A small number of rats used for special experiments as described in section (E), were bred in the laboratory. The animals were under preliminary observation for at least one week, usually three weeks, and if there was no obvious evidence of disease they were then put under the experimental conditions.

Clinical Notes.—The rats were active, displayed considerable interest in life, especially during the feeding hour, and ate with avidity. Their fur was glossy, the animals felt sleek and supple to the touch, and their dispositions were on the whole good. The average time under observation was eight weeks. Weights and temperatures were taken once a week, unless for some special purpose it was deemed advisable to make the readings more frequent. The observations were made as far as possible under the same conditions.

During the course of this work, two rats developed "head-trouble," which also occurred in several rats on other diets, due to acute suppurative mastoiditis, sometimes bilateral: occasionally this went on to meningitis and cerebellar abscess. The symptoms always commenced with the rat keeping its head on one side. In the most advanced condition the rat keeps rolling over and over and makes desperate attempts to clutch the cage wall so as to keep itself in one position.
In fig. 1 is seen the temperature chart made from the compiled averages of 20 unselected rats (10 bucks and 10 does) on P diet. The upper dotted line represents the upper extremes in temperature and the lower dotted line the lower extremes. There is a great deal of individual variation some of which no doubt is due to pathological lesions.

In fig. 2 is seen the weight curve made from the compiled averages of 20 unselected male rats on P diet. There is a steady increase in weight.

*Morbid Anatomy.*—With the exception of the two cases of mastoiditis referred to, not one of the 48 "normal" rats autopsied showed clinical evidence of morbidity. After the animals had been under observation for the desired length of time, they were killed in the manner already described and the tissues were generally in the fixative within fifteen minutes after death.
In table II. are shown the gross autopsy findings on the contents of the alimentary tract of these 48 rats. I have deliberately gone into some detail in this table in order to demonstrate the great individual variations and also to serve as a base line for observations which are to follow. It will be seen that the gross anatomical findings in this respect do not differ very widely between the averages of 48 unselected cases and the average of 16 normal rats which showed at autopsy no gross evidence of abnormality in the organs. Two rats showed a thickened cecum to one of which there was an adhesion of the meso-testis of the same side.

**Table II.**

Gross autopsy findings in the alimentary tract with reference to its contents, expressed as per cent. of incidence of arbitrary values.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45 unselected cases</td>
<td>16 selected cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>Distended</td>
<td>17</td>
<td>0</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>53</td>
<td>82</td>
<td>79</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Almost or completly empty</td>
<td>30</td>
<td>18</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Duodenum</td>
<td>Distended</td>
<td>14</td>
<td>20</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>88</td>
<td>80</td>
<td>85</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Distended</td>
<td>15</td>
<td>28</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>89</td>
<td>66</td>
<td>79</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>11</td>
<td>33</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Ileum</td>
<td>Distended</td>
<td>6</td>
<td>0</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>88</td>
<td>94</td>
<td>82</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Coecum</td>
<td>Distended</td>
<td>22</td>
<td>28</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>60</td>
<td>61</td>
<td>74</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>9</td>
<td>11</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>Distended</td>
<td>35</td>
<td>56</td>
<td>64</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>37</td>
<td>22</td>
<td>27</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>28</td>
<td>22</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>12</td>
<td>6</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Descending colon</td>
<td>Distended</td>
<td>28</td>
<td>50</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Moderately full</td>
<td>24</td>
<td>28</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>50</td>
<td>22</td>
<td>20</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

31 per cent. of lungs showed evidence of gross inflammation. The thyroid showed a moderate variation in size but otherwise no gross lesion. The retro-ileocecal glands were markedly enlarged in 7 per cent. of cases and moderately enlarged in 30 per cent. For the rest, several livers showed an occasional small yellowish nodule which
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turned out on microscopic examination to be a localised granuloma resembling a gumma. An occasional cyst of *Taenia crassicollis* was also seen. The livers and spleens varied somewhat in size but were otherwise apparently normal. The kidney and heart showed no gross lesions. The adrenals are considered in detail in a separate publication (21). The thymus showed some variation in shape but was on the whole of good size. The intestine of 9 per cent. of these rats contained the flat worm *Hymenolepis murina*.

*Microscopically* the following organs were examined: thyroid, parathyroid, thymus, heart, lungs, liver, spleen, kidney, adrenal, testis, vesicule seminales, ovary, retro-ileocecal glands, mesenteric glands, striated muscle, first part of duodenum, mid-jejunum, terminal ileum, ileocecal junction, first part of ascending colon, descending colon just above the brim of the pelvis. In all, some 2600 slides were examined. Tables III to VIII summarise the results in contrast with the experimental tissues. It is evident that there is hardly a lesion produced in these experimental animals which is not found in some of the normals.

**TABLE III.**

*Microscopic findings in the thyroid, expressed as per cent. of incidence.*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Interlyc[ ]olar cells</th>
<th>Alveolar epithelium</th>
<th>Size of alveoli</th>
<th>Corpora amylaceae</th>
<th>Congestion</th>
<th>Fibrosis</th>
<th>Acute inflammatory foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Very few.</td>
<td>Moderate</td>
<td>Very many.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Very few.</td>
<td>Moderate</td>
<td>Very many.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Very few.</td>
<td>Moderate</td>
<td>Very many.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Very few.</td>
<td>Moderate</td>
<td>Very many.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The great variability of the thyroid has been pointed out by many investigators but it is to be noted that 4 per cent. of my “normal” rats showed acute inflammatory lesions, and 12 per cent. showed distinct evidence of some fibrosis (table III).

**TABLE IV.**

*Microscopic findings in lungs, expressed as per cent. of incidence.*

<table>
<thead>
<tr>
<th>Diet</th>
<th>No evidence of lesion</th>
<th>Some evidence of pathological lesion</th>
<th>Active inflammation</th>
<th>Completely healed conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>35</td>
<td>65</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>100</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>B</td>
<td>58</td>
<td>42</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>74</td>
<td>26</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

JOURN. OF PATH.—VOL. XXVII.
In the gross anatomical inspection, 31 per cent. of rats showed some lung lesion. The microscope, however, revealed that 65 per cent. of my control rats showed some evidence of inflammation (table IV.). In 39 per cent. the lesions were evidently healed. The remaining 26 per cent. showed conditions which ranged from bronchiectatic abscesses to cheesy granulomata. In some the lesions were small and localised, in others lobular, and in several lobar. It is to be noticed that without the microscopic examination, 69 per cent. of my rats would have been passed as normal in so far as the lungs were concerned.

**Table V.**

*Microscopic findings in the liver, expressed as per cent. of incidence.*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Some congestion.</th>
<th>Very marked congestion.</th>
<th>Hyaline appearance of cells.</th>
<th>Inflammatory or necrotic foci.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>65</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>A</td>
<td>79</td>
<td>54</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>B</td>
<td>48</td>
<td>14</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>79</td>
<td>7</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

23 per cent. of the livers show inflammatory foci (table V.). These varied from slight focal polymorphonuclear accumulations to degenerative necrotic foci and to distinct granulomata. The lesions in the liver gave me the impression of being metastatic embolic infections of the same nature as those which involved the lungs. Whether these were due to retrograde emboli from the lungs, or to clumps of bacteria which had passed the systemic circulation and been caught in the liver, or finally to independent foci from an intestinal lesion, I cannot say. The latter source, however, was suggested very strongly in a number of cases where the liver lesions seemed to bear a relation to the extent of the intestinal granulomatous ulcers which, as will be seen in table VIII., were found in the ileocecal region of 19 per cent. of control rats.

**Table VI.**

*Microscopic findings in the kidney, expressed as per cent. of incidence.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>21</td>
<td>26</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>A</td>
<td>36</td>
<td>71</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>33</td>
<td>15</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>33</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>
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The fixative which I used is particularly bad for the kidney so that it was difficult to define artefact from genuine lesion. Nevertheless, 6 per cent. of rats showed cloudy swelling, and in 21 per cent. casts were seen in the collecting tubules: these were usually hyaline (table VII).

The spleen (table VII.) is a notoriously variable organ, and I have shown elsewhere(6) that it is unwise to draw conclusions from its histology unless similar age periods are being compared. Since my rats were more or less of the same age there will be some significance in the changes found.

TABLE VII.

Microscopic findings in the spleen, expressed as per cent. of incidence.

<table>
<thead>
<tr>
<th>Dist.</th>
<th>Pulpatrophy</th>
<th>Well marked increase of pigment</th>
<th>Bloody.</th>
<th>Thickened and hyaline blood vessels</th>
<th>Prominence of trabeculae</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>31</td>
<td>23</td>
<td>54</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>67</td>
<td>66</td>
<td>83</td>
<td>33</td>
</tr>
<tr>
<td>B</td>
<td>72</td>
<td>28</td>
<td>86</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

18 per cent. of the control rats showed a moderate vacuolation of the cells of the pancreas. 5 per cent. of cases show acute inflammatory infiltrations and 3 per cent. sclerosis of the pancreas. In no case was a lesion of the islets of Langerhans found.

The testes of 17 per cent. of the rats presented here and there tubules which showed faulty spermatogenesis and irregular arrangement of the germinal cell layers. The bulk of the tissue, however, appeared quite normal. In 100 per cent. of the vesiculae seminales the lining epithelium was cylindrical. The vesicles were well filled with secretion. In 24 per cent. of the ovaries, occasional ova showed degenerative changes, the majority of the Graafian follicles, however, were normal.

TABLE VIII.

Per cent. incidence of inflammatory lesions in the cecal aspect of the ileocecal region.

<table>
<thead>
<tr>
<th>Dist.</th>
<th>Ulceration</th>
<th>Distinct inflammatory infiltration</th>
<th>Slight accumulation of inflammatory cells</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>19</td>
<td>12</td>
<td>30</td>
<td>61</td>
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<tr>
<td>A</td>
<td>18</td>
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<td>59</td>
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<td>B</td>
<td>13</td>
<td>11</td>
<td>16</td>
<td>40</td>
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<tr>
<td>C</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>
By far the most marked intercurrent intestinal lesion was seen on the cecal aspect of the ileocecal region (table VIII.). Here, in 61 per cent. of the cases, collections of inflammatory cells were found in varying grades of severity. In 30 per cent. this was mild and consisted of localised accumulations of polymorphonuclear leucocytes and plasma cells lying on the muscularis mucosae and immediately beneath it. In 12 per cent. of cases this lesion was more severe and extensive, forming in fact a well-marked and quite evident infiltration. From this, all grades of extension and severity led up to conditions of marked ulceration. The base of these ulcers consisted of markedly fibrosed granulomatous tissue covered with a layer of necrotic debris and thickly infiltrated with cells of acute inflammation. This lesion frequently extended through all the coats of the intestine locally. Ulceration occurred in 19 per cent. of control animals. It is perhaps no coincidence that 61 per cent. of control animals showed some form of localised cecal inflammation and that 65 per cent. of the same animals showed some evidence of a lung lesion. The two conditions were usually found in the same rat.

Moreover, after some experience the cecal inflammation could be anticipated by inspection of the retro-ileocecal glands. These glands were found enlarged under 75 per cent. of the ulcers. Besides these ulcers, 25 per cent. of the other inflammatory foci showed enlarged glands. In only 6 per cent. of cases which showed enlarged retro-ileocecal glands did I fail to find a microscopic inflammatory focus in the cecum. The main cause of the enlargement of the gland is lymph stasis. Although over 20 per cent. of the glands showed some evidence of inflammation, this was in no way commensurate with the extent of the intestinal lesion.

The remainder of the intestine showed few changes. These are best considered when dealing with the results of vitamin deficiencies.

It may be argued that my control animals are so grossly abnormal that they can hardly serve as a base line, but, as I have stated earlier, though my rats were by no means of especially selected stock, they represent the sort of animals used in most experiments of the kind now being carried out on the effects of vitamin deficiencies on adult animals. Moreover, I took reasonable precautions to determine clinically whether the rats were abnormal, and it was only at autopsy and upon careful microscopic examination that the lesions described were found.

It is my intention to make a similar statistical examination of adult rats bred under my observation. I have no doubt that the incidence of morbidity will be lower, perhaps considerably lower, but the point which I wish to make is that unless gross and histological examinations are carried out in detail and on a large number of control animals, the experimental results lose much of their validity. Perhaps this point seems to be so obvious as to require no emphasis, but I
have found on the whole that the control animals used in a considerable
number of experiments quoted in the literature are either of an
astonishingly healthy stock or else they have not had as searching
an examination as my results indicate to be necessary.

We are now in a position to examine the lesions produced by
feeding vitamin deficient diets to rats.

(D) Observations on rats fed on vitamin deficient diets.

Studies on the pathology of vitamin deficiencies have largely
been in two specialised directions, viz., rickets and beriberi. As
neither of these questions concerns us here, I shall make no attempt
to review the enormous literature. Accounts of the pathology based on
only a small number of animals are unfortunately of little value, and
I must leave out of account those researches on the effect of diets
unbalanced in other respects besides their vitamin content, as well
as those whose microscopic results were obtained from tissues removed
from animals which were brought to the point of death on the experi-
mental diet. This leaves the present field of investigation practically
untouched: I shall, however, mention several isolated papers of special
interest in relation to the topics under discussion.

Clinical notes.—The 19 rats fed on A diet behaved on the whole
like normal rats. It was only in the latter weeks of the experiment
that they were noticeably affected with "colds" in a number of cases.
As a general rule the food intake was fair, compared to the normal.
Their average weights (fig. 2) showed a flattening of the growth curve.
After the fourth week there was a distinct drop. The temperature
chart (fig. 1) shows no significant difference from the normal. The
average length of time during which the rats were on this diet was
six weeks.

The 34 rats on B diet showed the most marked clinical manifestations
of vitamin deficiency. They were rarely kept on this diet longer than
six weeks, for it was my object to avoid pronounced disturbances which
cause a general metabolic upset with secondary phenomena. The
average time on this diet was five weeks. The first thing noticed was
the refusal to eat food, which may occur immediately, certainly within
a few days. Almost as striking is the loss in weight which commenced
soon after the inception of the experiment (fig. 2). This is, however,
not directly due to the lowered food intake, for even if the food con-
sumption is increased, as by addition of flavouring agents to the diet,
no growth results in the absence of vitamin B (Drummond 7). The
temperature suffers a gradual decline, and in about three to four weeks
is well below the normal (fig. 1). By this time the rat has usually
become so thin that its ribs are easily felt. The fur becomes shaggy,
the hair standing on end. The rat assumes a rather characteristic pose,
sitting with its back hunched up, and the slightest unusual sound
causes it to start or jump. If the diet is kept on, the rat develops
great weakness of the hind limbs, extreme emaciation, and incoordination. If the balance of the rat is disturbed as by swinging, and it is then placed on the floor, it rolls over and over helplessly for a time. The clinical picture of vitamin B deficiency in adult rats is thus totally different from that produced by deficiency of vitamin A and, as will be seen, of vitamin C. One of the vitamin B deficient rats developed acute suppurative mastoiditis.

The 20 rats on C diet exhibited clinically no characteristic difference from the normal. Their temperatures were on the whole somewhat higher than normal (fig. 1). The weight curve follows very closely the normal for the first few weeks on the diet; later it flattens out and drops (fig. 2). The average length of time during which the rats were on this diet was seven weeks. Because rats do not display the florid picture of scurvy which is so characteristically shown by guinea-pigs on vitamin C deficient diet, it has been held by some observers that rats require no vitamin C in their diet. Harden and Zilva (8) as well as Drummond (9) are, however, of the opinion that rats which are deprived of vitamin C do not thrive. This I can confirm for adult rats.

Morbid anatomy.—The thyroid, heart, kidney, and striated muscle showed more or less the same variability in the three vitamin deficiencies as in the normal. The livers of vitamin B deficient rats decreased in weight, the decrease being fairly proportional to the length of time on the diet. The incidence of small spleens was considerably greater than normal in the three vitamin deficiencies, being greatest in vitamin B deficiency, next in C and last in A deficiency. The latter diet showed the greatest incidence of large spleens, probably on account of the greater incidence of infection. The thymus showed the most striking change, presenting invariably a marked atrophy in vitamin B deficiency which has already been pointed out by numerous observers.

The lungs showed gross lesions in 63 per cent. of vitamin A deficient rats, 23 per cent. of B deficient, and 42 per cent. of C deficient animals, compared with 31 per cent. in the controls. As opposed to 9 per cent. incidence of flat worms (usually *Hymenolepis murina*) in normal rats, 22 per cent. of vitamin A deficient rats, 18 per cent. of B deficient rats, and 30 per cent. of C deficient rats harboured these parasites in their intestines. Enlarged retro-ileocolcal glands were found in 37 per cent. of control rats, 23 per cent. of vitamin A deficient rats, 38 per cent. of B deficient rats, and 20 per cent. of C deficient rats.

Table II. brings out some points in the gross content of the intestinal tract which are of interest in connection with some *in vivo* experiments on motor function which will be described below. It is seen that the overdistended stomach so often attributed to vitamin B deficiency is in fact found more frequently in normal rats, as is to be expected from their higher food intake.

Peyer's patches were less prominent in vitamin B deficient rats,
otherwise the small intestine showed immediately after death on the whole little difference from the normal; the possible exception being the somewhat higher incidence of gas in the intestine of vitamin A deficient rats. It is necessary to emphasise the words “immediately after death,” because even in half an hour fermentation set up in the intestinal contents produces often in vitamin A and probably also in B deficient animals more ballooning than is seen in normal rats. This is probably due to differences in bacterial flora or in thickness of intestinal musculature or in both. As will be shown later, evidence is adduced which suggests a thinning of intestinal muscles. Two rats on vitamin C deficiency presented thickened ceca. This was also found in one vitamin A deficient rat where it was the seat of an adhesion to meso-testis of the same side. Otherwise, with the exception that the cœcum of vitamin B deficient rats was never found empty or small, this viscus showed on the whole little gross difference from the normal. The ascending colon showed a considerable increase in incidence of distension in vitamin B deficient rats and to a lesser extent in vitamin A deficient rats. The descending colon showed in both vitamin A and B deficiency, a considerably greater incidence of distension with faeces. 50 per cent. of control rats and 65 per cent. of C rats showed a completely empty descending colon. This was empty in only 22 per cent. of vitamin A deficient rats and in 20 per cent. of B deficient rats (see p. 43 below).

Gross observations on the alimentary tract show, therefore, no striking differences between normal and vitamin deficient rats. When, however, the motor functions are studied a marked difference is seen.

Microscopic examination showed some increased incidence of cells lying between the alveoli of the thyroid gland in vitamin B deficiency (table III.). The cells are probably thyroid epithelial cells which are not arranged in glandular form. Vitamin B deficiency is also associated more frequently than normal with somewhat larger and more distended alveoli. Otherwise the variations in microscopic appearance of the thyroid gland from rats on the various vitamin deficiencies differ in no essentials from the controls. This is also true for the parathyroids.

Table IV. brings out the striking fact that vitamin A deficiency presented in every case some form of lung lesion. Of these 74 per cent. were active acute conditions. This is in sharp contrast to the other vitamin deficiencies. The microscopic appearances of these lesions were on the whole similar to those already described for the control rats.

The interesting points to be noticed in the liver (table V.) are the great congestion found in vitamin A deficiency and the hyaline appearance of the cells frequently found in vitamin B deficiency. It is possible that the latter appearance is due to the low store of
glycogen which P. Eggleton and I have found associated with this condition. There is a slightly higher incidence of inflammatory foci in the livers of vitamin A deficient rats.

The kidneys (table VI.) showed a considerable increased incidence of vacuolation of the convoluted tubules nearest the pyramids also in the more marked congestion in vitamin A deficiency. Cloudy swelling was most frequently seen in the kidneys from vitamin B deficient rats.

Bloody atrophic spleens (table VII.) are encountered most frequently in vitamin B deficient rats. The atrophy involves chiefly the pulp cells and not, as has been stated, the lymphoid tissue. The spleens of vitamin A deficient rats showed a huge increase of thickened hyaline blood vessels and of pigment. The findings in the spleen must however be taken with great caution on account of the great changes which the constituents of the spleen undergo with age (Gross 6).

The pancreas showed a greatly increased incidence of vacuolation on vitamin B deficiency (63 per cent. as opposed to 18 per cent. in the control rats). The vacuoles were usually much more prominent as well. Vitamin C deficiency showed a vacuolation in 43 per cent. and A deficiency in 21 per cent. No lesions were found in the islets of Langerhans in any of the vitamin deficiencies. Some observations on the carbohydrate metabolism will be found elsewhere (20).

All three vitamin deficiencies showed an increased incidence of atrophy in the testes (60 per cent. in vitamin A deficiency, 61 per cent. in B, and 50 per cent. in C, compared with 17 per cent. in controls). This atrophy varied in degree from a marked shrivelling up of the seminiferous tubules with almost complete disappearance of cellular contents to disorganisation of the cell layers with cessation of spermatogenesis, as first described by Drummond (7). A curious finding, most frequently seen in vitamin B deficiency, is the presence in the tubules of large spherical giant cells with many small clear bladder-like nuclei. The vesiculae seminales showed in all three deficiencies a flatter epithelium. In vitamin B and C deficiency the epithelium frequently showed pyknotic nuclei surrounded by a clear zone.

The ovary showed in all three deficiencies a considerably higher incidence of Graafian follicles undergoing complete degeneration (80 per cent. in vitamin A deficiency, and 75 per cent. in B and C, as opposed to 24 per cent. in controls). Frequently all that remained of the follicle was a narrow layer of the stratum granulosum, the cavity being filled with necrotic debris.

The findings in the intestine were on the whole rather disappointing. A moderate atrophy of the reticular cells of the mucosa was best seen in the descending colon of vitamin B deficient rats (43 per cent. as opposed to 6 per cent. in controls). In the duodenum of 2 per cent. of control rats a more or less granular exudate (or transudate)
containing occasional lymphocytes was seen lying immediately under the epithelium of the tips of the villi. This condition was seen in 16 per cent. of vitamin B deficient rats, in 13 per cent. of A deficient rats, and in 5 per cent. of C deficient rats. It is interesting to note that a few pigment cells resembling those found in the human colon are seen in the ascending colon of the rat. These lie, however, under the muscularis mucosae and occur only in 2 per cent. of control rats, 6 per cent. of vitamin deficient rats, 3 per cent. of B rats and in none of the C deficient rats examined microscopically. Ulceration was found in the ascending colon of 6 per cent. of control rats and 3 per cent. of vitamin B deficient rats. The ileocecal region showed lesions similar to those already described in the control rats, and in approximately the same proportion of incidence. The only interesting point noticed in the microscopic sections of the retro-cecal glands was the increased incidence of pigment-bearing cells in the sinuses of the glands from vitamin B deficient rats. It is to be remembered that cells somewhat similar in appearance are found in the colon from cases of stasis in the human. These vitamin B deficient rats suffer, as will be seen later, from considerable stasis.

In 16 per cent. of vitamin B deficient rats the Peyer's patches were seen to undergo regressive changes. These consisted in elongation and clumping of the lymphocytes which stained very deeply with haematoxylin. Hyaline connective tissue strands were seen between these clumps. Lesser grades of this lesion were encountered in 2 per cent. of control rats. With regard to Auerbach's plexus, I must confess that I have not been able to confirm M'Carrison's observations. With the fixative and stains which I employed, it was impossible to determine how much of the disorganisation found was due to artefact and how much was actually a pathological lesion.

The lesions specific to vitamin deficiencies which I found in the adult rats are thus seen to be extremely few. Atrophy of the thymus in the case of vitamin B is the only special lesion. The other changes, such as atrophy of the spleen pulp, slight atrophy of sub-epithelial intestinal reticularis, etc., are found in a certain proportion of control animals and show an increased incidence in the various vitamin deficiencies. The rather marked vacuolation so frequently seen in the pancreas of the vitamin B deficient rat is worthy of note and undoubtedly has some bearing on the function of the organ under these conditions.

Cramer (10) has recently called attention to the extreme atrophy which occurs in the intestine of vitamin A deficient rats. I can only assume that the discrepancy in our results is due either to the fact that the rats which he employed were young and growing, whereas mine were adult, or to a difference in the length of time on the diet.
(E) Investigations on the effects of vitamin deficient diets on the motor functions of the intestinal tract *in vivo* and *in vitro*.

The *in vivo* studies on the effect of vitamin deficiencies were carried out on animals which I had bred in this laboratory. In every case the control experiment was carried out on the same animal, and at the end of the experiment each rat was killed and autopsied in order to rule out intercurrent lesions.

The method which I finally adopted was to use rats weighing approximately 100 grams. These were given a small ball of diet weighing 1 gram with which 5 milligrams of finely-powdered animal charcoal were thoroughly mixed. The animal was then placed on a specially designed metabolism apparatus (Gross and Connell 11) which automatically times and separates the excreta. With this apparatus it was possible to estimate to within an hour the time taken for the first appearance and for the disappearance of the charcoal in the feces. It is essential to use a weighed amount of charcoal, for Connell and I have found that the time taken for the charcoal to disappear apparently varies, in the same animal, directly with the amount given. The first appearance, however, is independent of this factor. Another point to be noticed is that the length of time which it takes for the last trace of charcoal to disappear from the feces varies inversely more or less with the age, or rather size, of the animal. That is to say, for the same amount of charcoal, a smaller animal takes longer to free its intestine from the last traces than a larger one. For this reason, it is important to sandwich the experiment in between two control experiments.

The first appearance of charcoal is usually sharp and made out with very little difficulty. The end point is somewhat more difficult to determine. If there is any doubt as to whether the colour of the scybalon in question is due to bile or to charcoal, it is a good plan to receive the feces into vessels containing a small quantity of water. This keeps them moist and soft until it is desired to examine them. If the scybalon is smeared over a piece of rough white paper, the black mark left by charcoal is easily distinguishable from the bile stain. If there is further doubt, the pellet may be mushed up in a test tube containing several cubic centimetres of 25 per cent. sulphuric acid. This digests and floats to the surface the main constituents of the feces without charring them and allows the charcoal to settle as a distinct black deposit.

The observations recorded here are based upon the results of investigations on 18 rats which were found normal at autopsy. The length of time necessary for the first appearance and disappearance of charcoal while the animal is on P diet was found for each rat; this was then determined for the experimental diet, and finally for P diet again. The vitamin deficient diets tested were A and B. C diet was not tried as time did not permit.

The average time taken for the charcoal to make its first appearance was approximately ten hours for P as well as for the vitamin deficient diets. The time taken for the charcoal to disappear completely
was enormously increased in the case of vitamin B deficiency. It was considerably shortened in vitamin A deficiency. Two typical examples will illustrate this point.

Rat 390 on P diet took nine hours to show the first trace of charcoal; in sixty-five hours this had entirely disappeared from the faeces. The same rat on B diet showed the first appearance of charcoal in twelve hours; after sixteen days the faeces still showed a considerable amount of charcoal and it was only after administering “marmite” for eight days that the last traces of charcoal disappeared. The rat was then fed on P diet for a day and tested: the charcoal first appeared in ten hours and disappeared in five days. Fig. 3 shows
this result diagrammatically. It is noteworthy that this rat was by no means ill when it was first given B diet. Immediately after the first timing experiments on P diet were over, two days more on P diet were allowed to elapse after the last appearance of the charcoal, in order to make certain that the last traces had been emptied. The rat was then placed on B diet for one day, after which the second timing observations were made. The food intake of the rat was considerably lowered, especially towards the middle of the experiment. A change, too, was noted in the faeces, both in quality and quantity. They became large and puffy, and somewhat harder; at the same time there was a considerable diminution in their number, so that whereas this rat passed on an average thirteen to fourteen scybala a day while on normal diet, during the second week on B deficiency it passed on an average two a day. Occasionally, twenty-four hours or more elapsed without the passage of faeces, a very unusual occurrence for a rat.

Fig. 4 shows the effect of 0.2 grm. daily of "marmite" on this rat after the sixteenth day on B deficiency. B diet was continued and the amount given was deliberately kept the same as what the rat had been taking daily during the B deficiency. Nevertheless, as is seen, after a lag of three days the number of scybala passed daily rapidly increased until the former normal level was reached. To
determine whether the "marmite" had a local stimulating effect on the bowel, a fact which did not seem probable a priori since its effect became manifest only after three days, I tested the effect of isotonic and hypertonic solutions of this substance on the isolated rat's intestine. They produced an inhibition and fall in tone even in so low a concentration as 1 in 150,000. It might be argued that the stasis produced by vitamin B deficiency was more apparent than real, and that the delay in disappearance of the charcoal was due to diminished intake with consequent diminished output. This, however, while it may explain the smaller number of scybala passed, does not account for the delay in expulsion of the intestinal contents such as they are. Moreover, as I have shown, the simultaneous administration of "marmite" to the same rat on the same diet kept to the same quantity as the intake while on B deficiency counteracted the delay in the expulsion of the intestinal contents as evidenced by the charcoal. Finally, the action of the "marmite" is certainly not a local one for, apart from its inhibitory effect on the isolated intestine and the comparatively long time (for a laxative) before its effects were produced, it caused no diarrhea in rats fed on either normal diet or B diet. The salt content of the 10 per cent. solution which I actually administered by mouth was under 3 per cent. It seems to me, therefore, that these experiments prove that vitamin B is essential for the maintenance of the normal motor functioning of the intestine. I have no doubt that in the normal economy of the bowel other factors, such as roughage, also play an important part, for in some timing experiments in which I studied the effects of powdered agar I found that this indigestible material does stimulate the activity of the bowel. Furthermore, in studying the effects of such foods as carrots, I found that the speed with which this food passed through the alimentary tract suggested that other directly stimulating substances besides roughage and vitamins may also serve as exogenous regulators of intestinal activity. Autopsies performed on rats killed during stasis showed the charcoal accumulated in the cecum, which with the colon (section D above) is probably the first to suffer from the result of vitamin B deprivation. The most remarkable point is, that the intestine seems to feel the need of vitamin B almost immediately, thus bearing out the conclusions of previous observers as to the inability of higher organisms to store this vitamin in appreciable quantity. In this connection, it is interesting to note that brewer's yeast is used as a laxative by the farmers in the North of France (Herbomez 12). Reeves (13) working on six cases of constipation in human subjects was able to cure four and relieved the remaining two by the administration of yeast. Smith (14) found that when human subjects are kept on a diet of bread, milk, and cheese, they have a tendency to become constipated. Adding yeast to the diet, however, relieves the constipation. Recently Murlin and Mattill (15) have
reviewed the literature on the subject and performed a number of experiments on the laxative properties of yeast using human subjects and dogs for the purpose. Their criteria of constipation were increased water content, bulk, and nitrogen content of the stools. They found that boiled yeast had a weaker laxative action. It seems to me that the rate with which the intestine clears itself of a given amount of inactive pigment is a much more certain measure of the motor activity of the bowel, and that the experimental results which I have produced can hardly be called by any other name than "constipation." I am using this term to indicate a symptom which I am quite well aware may have its origin in a variety of causes. The results obtained by the use of yeast, although not so clear cut as the experiments which I present here and more open to criticism, nevertheless bear out my contention that the lack of vitamin B is followed by a stasis in the alimentary tract, and that a stasis so produced is relieved by the exhibition of vitamin B.

Rat 396 illustrates the effect on the motor functions of the intestinal tract of the deprivation of vitamin A. This rat was somewhat younger, hence took six days to empty the bowel of the last trace of charcoal in the P diet. It took six hours for the first appearance. Two days after the last appearance of charcoal, A diet was given and the following day timing observations were commenced. The charcoal appeared in five hours and disappeared in sixteen hours. Fearing that this was due to some error in technique the timing was repeated on A diet. This time it took twenty-three hours for the charcoal to disappear. A subsequent test with P diet in the usual way showed a first appearance of charcoal in nine hours and a disappearance in eight days (fig. 3). The faeces remained more or less constant in number, or perhaps in several cases showed a slight increase. This was repeated on a number of rats.

In both A and B deficiencies, particularly in the former, the faeces were grey and somewhat lacking in bile pigments; this was more noticeable in comparing the dried scybala. That food rich in vitamin B contains substances which stimulate the flow of bile and pancreatic juice was shown by Voegtlin and Myers(16). It is seen, therefore, that vitamin A deficiency, although undoubtedly associated with an alteration in the secretory functions of the alimentary tract, caused no initial stasis but rather a more rapid peristalsis.

The question which now arose was, "what structures in the intestinal tract are involved in this stasis?" I have shown that histological evidence on this point is very uncertain. I attempted no comparisons of the thickness of the muscle because this varies so enormously according to the state of tonus or stretching of the intestine when fixed. Such comparisons are only possible where differences are very marked. This was not the case in the sections which I examined.
I therefore attempted to determine whether there was any difference between the work done by the isolated intestine from vitamin deficient rats and that from the normal, estimating the work done in arbitrary dynamic units. I hoped that this would give me a clue as to the amount or state of the musculature.

The rat to be examined was killed in the manner described, the abdomen was immediately opened, slips of the first part of the duodenum, terminal ileum, and ascending colon (always taken at the same points) were removed, wrapped in gauze dampened with Ringer solution and placed on ice. This caused a tonic contraction of the smooth muscle. At fixed intervals thereafter, 4 cm. of the different portions of the intestine were removed and placed into a bath containing 250 c.c. of Tyrode's solution of tested Pₚ and kept at a constant temperature of 39°C. After an interval of fifteen minutes a tracing was taken of the rhythmic movements of the slip. No oxygen was used.

**Table IX.**

Units of work performed by unit portions of isolated small intestine from rats on the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Duodenum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet only</td>
<td>Diet and daily intraperitoneal pituitrin.</td>
</tr>
<tr>
<td>P</td>
<td>52.2</td>
<td>42.6</td>
</tr>
<tr>
<td>A</td>
<td>39.7</td>
<td>32.8</td>
</tr>
<tr>
<td>B</td>
<td>39.1</td>
<td>...</td>
</tr>
<tr>
<td>C</td>
<td>27.6</td>
<td>34.5</td>
</tr>
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</table>

Several points in connection with this technique deserve mention. First, by placing the intestine on ice I subject each intestine examined to as nearly as possible the same degree of tonus. Measured sections under this condition are therefore fairly comparable providing that due allowance is made for the differences in thickness due to the differences in size of the animal. Secondly, by avoiding the use of oxygen, I eliminate the differences which alterations in the supply of oxygen cause (Gross and Clark ²). Thirdly, given the same leverage of the writing point and the same weight of the lever, the product of amplitude and frequency is a measure of the work done by the muscle. Assuming that this work varies, amongst other things, with the musculature, and assuming that the musculature is a function of the weight of the rat, I attempted to reduce the work done by the various intestines to the same proportion by means of a formula. Needless to say, this method can give no more than an approximation of the
differences of the actual work done, and differences to be significant must be fairly large. The formula \* which I used was:

\[
\frac{\text{amplitude} \times \text{frequency}}{\left(\frac{\text{highest body weight}}{100}\right)^2}
\]

Table IX. shows the factors obtained for the duodenum and ileum under the several experimental diets and conditions. The following points are brought out:

(a) The work factor for the duodenum is considerably lower in the vitamin deficiencies.

(b) Whereas the ileum work factor is lower than that of the duodenum in the control animals, this order is reversed in each of the deficient groups. This may have some bearing on Alvarez'\(^{(1)}\) theory of dynamic gradient.

(c) With the exception of the ileum in vitamin A deficient rats, daily intraperitoneal injections of pituitrin caused no obvious difference in the work factor.

(d) Daily intraperitoneal injections of adrenalin were associated, contrary to what was expected, with an increase in the work factor which was astonishingly high in vitamin B deficient rats.

The only other observation which might be of interest in this connection is the greater and more rapid response which the duodenum and ileum of a number of vitamin B deficient rats gave to a concentration of 1 in 500,000 pilocarpine nitrate in the bath, as compared to that given by other vitamin deficient and normal rats.

The colon, on account of its irregular and sluggish motility, did not lend itself to a study of this nature.

If these experiments are a true index of the work power of the intestinal muscle, one may be allowed to conclude that vitamin

\* The internal and external diameters of the gut, it is assumed, vary roughly as the diameters of the rat. Further, the power of the muscle will depend on the volume of muscle employed in the experiments, other things being equal, such as stretching, temperature, etc. This volume will be given by the product of length and cross-section. The cross-section will be given by \(\pi (r_1^2 - r_2^2)\), where \(r_1, r_2\) are internal and external diameters of the gut respectively; this expression is a linear function of the square of the diameter of the rat. The length of the gut is constant (4 cm.), hence the volume of gut employed is proportional to the square of the diameter of the rat; the diameter of the rat, in turn, is proportional to the cube root of the rat weight. Hence the volume of the gut employed is proportional to weight \(\frac{2}{3}\). Each must be reduced to the volume corresponding to that given by a rat of unit weight (100 grms.). Hence the actual power measured by amplitude \(\times\) frequency must be divided by

\[
\left(\frac{\text{highest body weight}}{100}\right)^2
\]

The relative power is therefore given by

\[
\frac{\text{amplitude} \times \text{frequency}}{\left(\frac{\text{highest body weight}}{100}\right)^2}
\]
deficiencies on the whole diminish this power in the first part of the small intestine and alter the relations of the dynamic gradient. This must be an index of either atrophy or other qualitative changes in the muscular or neuromuscular apparatus.

(F) Conclusions.

The first point which deserves emphasis is the fact that apart from such specific lesions as rickets, beriberi, and scurvy, pure vitamin deficiencies produce in the adult rat remarkably few characteristic lesions. Similar conclusions have been arrived at by Simonnet (18) and Lumière (19) on pigeons, and by Karr (20) on dogs. If any conclusions which are to be applicable to man are to be drawn from dietary deficiencies in animals, the rat is not a very unsatisfactory animal inasmuch as its diet is very much of the same nature as that of man, and the anatomy of its intestinal tract shows an extraordinary resemblance to that of the primates. The results following a variation in the diet of the rat can, therefore, with more justification be applied to man than can those produced in birds, or even in herbivorous or graminivorous mammals.

I have attempted to show the advisability of performing autopsies on all cases where conclusions are to be drawn from dietary experiments on rats because these animals are very prone to morbid conditions. I have drawn attention to the necessity of performing histological observations on tissues taken from animals immediately after death in order that the results may be considered valid. Finally, I cannot commend too highly the practice of breeding one's own animals for experimentation.

The main object of this research was to determine whether or not pure vitamin deficiencies can be a source of intestinal stasis. This has proved positive for a deficiency of vitamin B. Although I have been unable to find unmistakable lesions in Auerbach's plexus produced by any of the vitamin deficiencies, the in vivo experiments showed a marked stasis immediately following the elimination of marmite from the diet. I have shown that marmite has no local stimulating effect on the isolated rat's intestine. Vitamin A deficiency produces an intestinal disturbance which manifests itself in a hurrying through of intestinal contents. Both of these vitamin deficiencies are accompanied by a qualitative difference in the feces. I have not studied the effects of vitamin C deficiency on the motor functions of the intestine of the rat in vivo.

Early in this paper I drew attention to the sub-epithelial accumulation of pigment-bearing cells which are characteristically found in great numbers in the colon removed at operation for conditions of advanced stasis. It is interesting to note that the retro-ileoceleal glands of vitamin B deficient rats alone showed a definitely increased incidence of pigment-bearing cells. Whether these cells are identical with those found in the human colon, I cannot at present say. It is
clear that the latter are the result of a chronic, long drawn-out condition, whereas those which I have found in rats occurred in relatively acute conditions of stasis.

Whether vitamin deficiencies play a rôle as an ætiological factor of some forms of intestinal stasis in everyday life, is not a matter which I desire to discuss here. Personally, I am inclined to believe that they do, and sufficient evidence has been produced in this paper to make the consideration of vitamin B deficiency an important factor in the prevention of this condition, especially since more attention is being paid to vitamins A and C as ætiological factors of morbid conditions met with in everyday life. If it should turn out that the present so-called vitamin B corresponds to more than one factor, as has recently been suggested by a number of observers, I cannot predict in which factor the "anti-stasis" element will be found.

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