THE PRODUCTION OF ARTERIOSCLEROSIS AND GLOMERULONEPHRITIS IN THE RABBIT BY INTRAVENOUS INJECTIONS OF DIPHTHERIA TOXIN.

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PLATES 14 TO 19.

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Attention has frequently been called to the presence of vascular lesions in man, especially fatty streaks in the aorta, following infectious diseases, such as diphtheria, scarlet fever, and typhoid. This association has naturally led to an assumption by many of an etiological relation between infectious diseases and arteriosclerosis and to frequent attempts to produce vascular lesions in animals by the injection of bacteria or their toxins. Since the limits of this paper do not permit of an extensive review of this subject brief mention only will be made of the more important work and that which seems to bear especially on the experiments here reported. Critical reviews of the subject have been made by Klotz (1, 2), Saltykow (3), Pickett (4), and Frothingham (5).

Gilbert and Lion (6) in 1889 traumatized the intima of the rabbit's aorta by passing a stylet down the carotid artery and afterwards injected cultures of B. typhosus. They thus obtained vegetative lesions. They also injected into two rabbits, without previous traumatization of the aorta, cultures of a bacillus obtained from a case of endocarditis. They produced plaques in the ascending portion and arch of the aorta, affecting mainly the media and consisting of fibrous tissue with some calcification.

Crocq (7) reported that traumatism of the aorta alone, by a method similar to that of Gilbert and Lion, produced no lesions; also that intravenous and subcutaneous injection of typhoid bacilli, colon bacilli, streptococci, and diphtheria bacilli, without previous traumatization, produced no lesions, but that when these bacteria were injected after previous injury to the aorta vegetations and plaques of endocarditis were produced. He therefore concludes that two factors are necessary, infection and a locus minoris resistentia.
Thérèse (8) and Pernice (9) injected Staphylococcus aureus, streptococcus, B. typhosus, B. diphteria, etc., into rabbits and guinea pigs without previous injury to the aorta. They obtained microscopical lesions only which consisted of round cell infiltration and later fibrosis about the vasa vasorum, affecting mainly the adventitia, but in the more severe cases extending to the other coats.

Boinet and Romary (10) also tried trauma combined with infection. They used various bacteria, including diphtheria bacilli and also filtrates of cultures. Raised yellow patches of small extent were produced in the aorta which sometimes did not correspond to the traumatized areas. Microscopically these consisted of nodules of infiltration about the vasa vasorum with overlying plaques of endarteritis.

Saltykow (3) by repeated injections of staphylococcus into rabbits obtained small plaques of fibrous thickening and fatty degeneration of the intima of the aorta and some of its main branches, and sometimes cellular connective tissue nodules with calcification in the upper media.

Klotz (1) reports the production, by the injection of B. typhosus and streptococcus, of wart-like thickenings involving the intima and inner part of the media of the first part of the pulmonary artery and ascending limb of the aorta. He also mentions, without giving details of his experiments, the production of a vascular lesion in the rabbit with diphtheria toxin. On this subject he says: "The repeated inoculations of diphtheria toxin into rabbits gave surprising results. Here, instead of meeting with proliferative changes, such as the B. typhosus and streptococcus produce in the aorta, there were only lesions of a degenerative character. The degenerations were isolated to the first part of the aorta, and were identical with those produced in the adrenalin series. The thinning of the arterial wall, with calcification and aneurysmal dilatations, were all present, and the microscopical examination showed the lesions to be confined to the media. No proliferative or inflammatory changes were present in the intima, nor was there any change about the vasa vasorum."

Frothingham (11) remarks the fact that none of the vascular lesions which have been experimentally produced with bacteria or their toxins in animals are of so severe a type as those seen in man, and with the object of studying the effect of diphtheria toxin on the arteries he injected nine rabbits subcutaneously or intravenously. Some of these showed partial necrosis of tufts of the glomeruli with fibrin formation and in cases also there was necrosis of the walls of some of the larger vessels in the kidney with deposition of fibrin. Capillary lesions were also present in the adrenals. In no case were lesions of any of the larger arteries observed. Only two, however, of the nine rabbits survived over 3 days. One of these had five injections at intervals of 11 to 18 days and was killed at the end of about 10 weeks. The other had four injections at similar intervals and was killed in about 7 weeks. Two guinea pigs were also studied which survived an injection of toxin and antitoxin for about a month. No vascular lesions were present. The author concludes that though the glomeruli and smaller vessels in the rabbit's kidney are susceptible to diphtheria toxin, and also the capillaries
in the adrenal, "the vessels of these animals, however, are not on the whole sensitive enough to this toxin to make it seem hopeful to secure permanent lesions by the administration of sublethal doses."

The experiments here reported were undertaken in the hope of producing an arteriosclerosis of the larger vessels of the rabbit with diphtheria toxin. Lesions of the small vessels in certain organs have been described by various authors, as by Babes (12), Welch and Flexner (13, 14), and Frothingham (11) in the kidney, and by Mollard and Regaud (15) in the heart. The attempts of the earlier workers, in some cases successful, as quoted above, to produce vascular lesions by trauma and infection combined leave one in considerable doubt as to the relative importance of these two factors in the production of the lesion. The lesions obtained by Thérèse (8) and by Fernice (9) with bacteria or their toxins were slight and hardly comparable in character, severity, or extent with those in man which are suspected by some observers of being due to a preceding infection.

EXPERIMENTAL.

With the exception of the observation of Klotz (1), with which, however, the writer was unacquainted until these experiments were practically completed, the previous work seemed to afford little hope of producing arteriosclerosis with diphtheria toxin alone. An incentive to attempt to produce vascular lesions by the combined action of increased blood pressure and a toxin was furnished by a theoretical discussion of the etiology of arteriosclerosis by Aschoff (16). A number of rabbits were consequently injected with diphtheria toxin alone, a second series with diphtheria toxin and pituitrin, and a number, as controls, with pituitrin alone. The pituitrin was obtained from Parke, Davis and Company. It was given always intravenously. For the rabbits in Table II, it was usually mixed with the diluted diphtheria toxin, but occasionally was given undiluted a few minutes after the intravenous injection of the toxin; to the rabbits in Table III it was given undiluted. Pituitrin was selected because it was believed that of itself it would produce no vascular degeneration. This view was confirmed by the control rabbits Nos. 33, 34, and 35. Adrenalin was unsuitable on account of the medial degeneration produced by it in the aorta, as first pointed out by Josué (17), and since confirmed by many others.

All rabbits were injected with a single sample of diphtheria toxin obtained from the Cutter Laboratories, dilutions of which, freshly made each day before injection with sterile 0.85 per cent salt solu-
tion, were of such strength that the total amount of the injection
was between 0.25 and 1 cc. The actual doses of undiluted toxin are
given in the tables. The 0.4 per cent tricresol, added to the toxin
by the manufacturers as a preservative, was disregarded under the
assumption that the exceedingly small amount injected was without
demonstrable morphological effect. All injections were made into
the ear vein. The doses of toxin, of toxin and pituitrin, and of pitui-
trin alone are given in Tables I, II, and III. All the rabbits died
from the effects of the treatment, except Nos. 33 and 34 which were
killed at the expiration of the intervals shown in the tables.

All the animals were carefully autopsied with special attention to
gross changes in the vessels. Frozen sections of various vessels and
organs were stained with Sudan III and hematoxylin and with Van
Gieson’s stain and hematoxylin. Paraffin sections were stained with
Van Gieson’s stain and hematoxylin and with eosin and hematoxylin,
and those of the vessels also with von Kossa’s stain for calcium.

TABLE I.

Rabbits Injected with Diphtheria Toxin.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Weight (gm)</th>
<th>Dose of diphtheria toxin (cc)</th>
<th>Total dosage (cc)</th>
<th>Duration of experiment (days)</th>
<th>Gross arteriosclerosis</th>
<th>Nephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,340</td>
<td>0.02</td>
<td>1.02</td>
<td>1½</td>
<td>Absent</td>
<td>Fatty degeneration</td>
</tr>
<tr>
<td>2</td>
<td>2,520</td>
<td>0.015</td>
<td>2.03</td>
<td>6</td>
<td>“</td>
<td>Acute glomerulo</td>
</tr>
<tr>
<td>3</td>
<td>2,470</td>
<td>0.006-0.007</td>
<td>4.025</td>
<td>4</td>
<td>“</td>
<td>Slight acute glomerulo</td>
</tr>
<tr>
<td>4</td>
<td>2,985</td>
<td>0.004-0.005</td>
<td>4.017</td>
<td>6½</td>
<td>“</td>
<td>Moderate acute glomerulo</td>
</tr>
<tr>
<td>5</td>
<td>2,240</td>
<td>0.003-0.004</td>
<td>3.013</td>
<td>9</td>
<td>“</td>
<td>Acute glomerulo</td>
</tr>
<tr>
<td>6</td>
<td>2,245</td>
<td>0.002-0.003</td>
<td>9.019</td>
<td>28</td>
<td>Present</td>
<td>Subacute glomerulo</td>
</tr>
<tr>
<td>7</td>
<td>1,640</td>
<td>0.002</td>
<td>5.01</td>
<td>5</td>
<td>Absent</td>
<td>Moderate acute glomerulo</td>
</tr>
<tr>
<td>8</td>
<td>1,655</td>
<td>0.002</td>
<td>5.01</td>
<td>5</td>
<td>“</td>
<td>“</td>
</tr>
<tr>
<td>9</td>
<td>2,260</td>
<td>0.002</td>
<td>4.008</td>
<td>8</td>
<td>“</td>
<td>Acute glomerulo</td>
</tr>
<tr>
<td>10</td>
<td>2,650</td>
<td>0.002</td>
<td>4.008</td>
<td>8</td>
<td>Present</td>
<td>Beginning subacute glomerulo</td>
</tr>
<tr>
<td>11</td>
<td>1,870</td>
<td>0.0015-0.0025</td>
<td>11.019</td>
<td>21</td>
<td>“</td>
<td>Moderate</td>
</tr>
<tr>
<td>12</td>
<td>1,670</td>
<td>0.0015</td>
<td>4.006</td>
<td>10</td>
<td>Absent</td>
<td>Beginning</td>
</tr>
<tr>
<td>13</td>
<td>1,880</td>
<td>0.001-0.002</td>
<td>17.0235</td>
<td>24</td>
<td>“</td>
<td>Moderate</td>
</tr>
<tr>
<td>14</td>
<td>2,010</td>
<td>0.001-0.0015</td>
<td>12.0135</td>
<td>20</td>
<td>Present</td>
<td>Subacute glomerulo</td>
</tr>
<tr>
<td>15</td>
<td>1,900</td>
<td>0.0003-0.001</td>
<td>17.0125</td>
<td>26</td>
<td>Absent</td>
<td>Slight subacute glomerulo</td>
</tr>
</tbody>
</table>
C. H. BAILEY

### TABLE II.

**Rabbits Injected with Diphtheria Toxin and Pituitrin.**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Weight (gm.)</th>
<th>Dosage of diphtheria toxin (cc.)</th>
<th>No. doses of diphtheria toxin</th>
<th>Total dosage of diphtheria toxin (cc.)</th>
<th>Pituitrin (cc.)</th>
<th>Duration of experiment (days)</th>
<th>Gross arteriosclerosis.</th>
<th>Nephritis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>2,010</td>
<td>0.03</td>
<td>1</td>
<td>0.03</td>
<td>2 doses of 0.4 cc.</td>
<td>12</td>
<td>Absent.</td>
<td>Fatty degeneration.</td>
</tr>
<tr>
<td>17</td>
<td>3,200</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>2 &quot; &quot; 0.7 &quot; &quot; 7</td>
<td>Present.</td>
<td>Acute glomerulonephritis.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>3,400</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>1 &quot; &quot; 0.7 &quot; &quot; 2½</td>
<td>Absent.</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>3,450</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>1 &quot; &quot; 0.7 &quot; &quot; 4</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2,640</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>1 &quot; &quot; 0.7 &quot; &quot; 2½</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2,900</td>
<td>0.015</td>
<td>1</td>
<td>0.015</td>
<td>3 doses &quot; 0.7 &quot; &quot; 2½</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2,860</td>
<td>0.01</td>
<td>2</td>
<td>0.02</td>
<td>4 &quot; &quot; 0.7 &quot; &quot; 3</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>2,545</td>
<td>0.006-0.007</td>
<td>4</td>
<td>0.025</td>
<td>3 &quot; &quot; 0.7 &quot; &quot; 5</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>2,600</td>
<td>0.005</td>
<td>2</td>
<td>0.01</td>
<td>5 &quot; &quot; 0.7 &quot; &quot; 5</td>
<td>&quot; &quot;</td>
<td>Moderate acute glomerulonephritis.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2,030</td>
<td>0.004-0.005</td>
<td>3</td>
<td>0.013</td>
<td>3 &quot; &quot; 0.7 &quot; &quot; 5</td>
<td>&quot; &quot;</td>
<td>Acute glomerulonephritis.</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>3,360</td>
<td>0.003-0.004</td>
<td>4</td>
<td>0.013</td>
<td>4 &quot; &quot; 0.7 &quot; &quot; 10</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>2,645</td>
<td>0.002-0.003</td>
<td>4</td>
<td>0.009</td>
<td>4 &quot; &quot; 0.7 &quot; &quot; 11</td>
<td>Present.</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1,465</td>
<td>0.002</td>
<td>5</td>
<td>0.01</td>
<td>5 &quot; &quot; 0.7 &quot; &quot; 4</td>
<td>Absent.</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1,575</td>
<td>0.002</td>
<td>5</td>
<td>0.01</td>
<td>5 &quot; &quot; 0.7 &quot; &quot; 9</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2,575</td>
<td>0.002</td>
<td>6</td>
<td>0.012</td>
<td>6 &quot; &quot; 0.75 &quot; &quot; 14</td>
<td>Present.</td>
<td>Beginning sub-acute glomerulonephritis.</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>2,240</td>
<td>0.0015-0.002</td>
<td>10</td>
<td>0.017</td>
<td>10 &quot; &quot; 0.75 &quot; &quot; 20</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1,615</td>
<td>0.0015</td>
<td>5</td>
<td>0.0075</td>
<td>5 &quot; &quot; 0.7 &quot; &quot; 9</td>
<td>Absent.</td>
<td>Acute glomerulonephritis.</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE III.

**Rabbits Injected with Pituitrin.**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Weight (gm.)</th>
<th>Pituitrin (cc.)</th>
<th>Duration of experiment (days)</th>
<th>Arteriosclerosis.</th>
<th>Nephritis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>2,260</td>
<td>4 doses of 0.5 cc.</td>
<td>4</td>
<td>None.</td>
<td>None.</td>
</tr>
<tr>
<td>34</td>
<td>3,110</td>
<td>23 doses of 0.5 to 1.0 cc.</td>
<td>23</td>
<td>&quot; &quot;</td>
<td>&quot; “</td>
</tr>
<tr>
<td>35</td>
<td>2 doses of 0.8 cc.</td>
<td>3</td>
<td>&quot; &quot;</td>
<td>&quot; “</td>
<td></td>
</tr>
</tbody>
</table>
In Tables I and II are also indicated the rabbits in which gross vascular lesions were found. A brief record of the vascular findings in these eight rabbits follows.

Rabbit 6.—The aorta is irregularly dilated especially in the thoracic portion. To the touch it is definitely stiffened. The large abdominal vessels, subclavians, and carotids are stiffened and the carotids show irregular dilatations. The aorta and carotids on opening show numerous transverse cracks and the lumen of these vessels is of varying diameter, an irregular thinning of the walls and shallow pouchings being present. Their walls are of the stiffness of parchment paper and many cracks are unavoidably produced by handling. The pulmonary artery is stiffened to its bifurcation. Sections of the vessels show a fatty degeneration of the smooth muscle of the media with crowding together of the elastic fibers into a compact layer. No calcification could be demonstrated.

Rabbit 10.—The aorta is irregularly dilated especially in the upper thoracic portion. To the touch it is stiffened throughout. The carotids are stiffened and of irregular diameter to the base of the skull. The subclavians, beginning of the large abdominal vessels, the iliacs, and about an inch of the proximal portion of the femorals are palpably stiffened. The aorta, on opening, has a scale-like appearance and shows shallow dilatations, and the abdominal aorta, carotids, subclavians, and iliacs show numerous transverse cracks. Sections of the thoracic aorta, abdominal aorta, and carotids show marked fatty degeneration of the smooth muscle of the media with crowding together of the elastic fibers into a compact layer. No calcification is present. There is considerable hemorrhage in the adventitia of the aorta.

Rabbit 11.—The thoracic portion of the aorta is slightly and irregularly dilated. The intima is of a dull gray color with fine closely set longitudinal strie. No lesions are apparent in the abdominal aorta or any of the other large vessels. Sections of the arch show necrosis of the muscle in the media without calcification. There is hemorrhage into the adventitia.

Rabbit 14.—The arch of the aorta shows a moderate irregular dilatation. The intima of the thoracic portion is whiter than normally and shows a definite irregular thickening with longitudinal white strie. The wall of the abdominal portion is stiff, and irregular areas of thinning are present. The carotids to the base of the skull, subclavians, iliacs, and the proximal inch of the brachials and femorals are slightly but definitely stiffened, of varying diameter, and on opening show numerous transverse cracks through the intima. Sections of the arch show an irregular marked cellular thickening of the intima without definite lesions in the media (Fig. 3). Sections of the abdominal aorta show a marked necrosis of the muscle of the media with crowding together of the elastic fibers, irregular thinning of the wall, and round cell infiltration in the adventitia. Sections of the carotid (Fig. 10) show necrosis of muscle, crowding of elastic fibers into a compact layer, and irregular thinning of the wall with small aneurysmal dilatations. No calcification could be demonstrated.
Rabbit 17.—An extreme diffuse lesion of the entire aorta from the aortic valves to the iliacs is present. The vessel is irregularly dilated, stiff, and definitely calcified throughout. A similar lesion involves the innominate, carotids, subclavians, large abdominal vessels, iliacs, and the brachials and femorals for a considerable distance. On palpation these vessels crackle under the finger like thin parchment paper and cracks are easily produced by bending. The pulmonary artery shows no gross lesion. Sections of the thoracic and abdominal aorta show a marked diffuse fatty degeneration and calcification of the media involving usually the inner third or half. The intima, and in places a thin layer of media overlying the zone of calcification, is necrotic and without calcium (Fig. 5). There is some hemorrhage into the adventitia. Sections of the carotids, iliacs, femorals (Fig. 11), etc., also show extensive necrosis and calcification of the media, generally diffusely involving the inner third, but in places extending nearly to the adventitia. The renal artery is similarly calcified to the kidneys. Small plates of calcification in the inner media of a large branch of the renal artery in the pelvis of the kidney are shown in Fig. 13.

Rabbit 27.—The lesions are of the same character, distribution, and severity as in No. 17, except that in this rabbit two small calcified plates, each about 3 mm. in diameter, are present in the pulmonary artery near the base. Sections of the arch (Fig. 4) and thoracic aorta show everywhere a wide zone of necrosis and marked calcification in the media. This calcified layer is of fairly uniform width in the arch but runs a wavy course, varying considerably in its distance below the intima. The elastic fibers themselves appear to be calcified. The intima and portion of the media overlying show considerable necrosis. In the abdominal aorta this overlying tissue, as well as the calcified material itself, in places, has sloughed off leaving deep ulcers (Figs. 7 and 8) which sometimes extend nearly to the adventitia, the latter showing hemorrhage and round cell infiltration. Small aneurysmal dilatations are numerous (Fig. 8). The carotids (Fig. 12) and iliacs also show extensive necrosis and calcification of the media.

Rabbit 30.—(Figs. 1 and 2.) The lesions are practically the same as in Nos. 17 and 27, but calcification of the iliacs is not so pronounced and is less extensive, and calcification of the brachials and femorals is not evident in the gross. The pulmonary artery shows a distinct fusiform dilatation (Fig. 1), the wall of which contains calcareous plates. The carotids are markedly stiffened to the base of the skull but no lesions of the vessels at the base of the brain, or in its substance, were evident in the gross or microscopically. Sections of the thoracic and abdominal aorta show similar widespread necrosis and calcification of the media with extensive and deep ulcers (Figs. 6 and 9), and hemorrhage and round cell infiltration in the adventitia. Sections of the smaller arteries also show extensive and practically uniform calcification of the media.

Rabbit 31.—The aorta on external inspection appears normal. The intima is whiter than normally and shows a slight but definite irregularly distributed thickening, more pronounced in the thoracic portion. The lower abdominal aorta for about 2 cm. above the iliacs is definitely stiffened and shows transverse cracks.
The pulmonary artery and branches of the aorta appear normal. Sections of the thoracic aorta show an irregular cellular thickening of the intima with no definite lesions in the media. Sections of the lower abdominal aorta show fatty degeneration of the muscular and elastic tissue of the media.

DISCUSSION.

The question at once arises whether the striking vascular lesion found in these rabbits is due to the treatment or preexisted as a spontaneous arteriosclerosis. That the latter occurs there can be no doubt, but in the author's experience it has been rare, only one case having been observed in a large number of rabbits whose vessels were carefully examined, and in this animal the lesion consisted of a single 2 mm. plaque of intimal thickening in the arch. Miles (18), however, reports the occurrence of spontaneous arteriosclerosis in seventeen out of forty-nine rabbits. In all these, however, the lesions were small and usually situated at the point of exit of a large vessel from the aorta. Whatever may be true as to the incidence of arteriosclerosis in rabbits no case has been reported, in so far as the author can discover, in which the lesions at all approximate in severity or extent those here reported. Even the vascular degeneration produced in rabbits by the administration of adrenalin chloride, first described by Josué (17), and since confirmed by others, but believed by some to be an independent and preexisting lesion, involves only the aorta and pulmonary artery in the experience of the writer, and it is believed that this accords with the observations of others; and even in the aorta the lesion is less severe and diffuse than that observed in these animals. The vascular lesion in these rabbits is so pronounced, in the peripheral vessels affected as well as in the aorta, that it is quite evident, after exposure of the vessel, on external inspection and even more so on palpation.

Several inconsistencies in the results obtained are, however, at once apparent on examination of the tables, as the presence of a pronounced arteriosclerosis in No. 14 and its entire absence in No. 13; also in Nos. 9 and 10. In order to produce the lesion it seems to be necessary to have a large amount of unbound toxin circulating in the blood and to produce this large doses must be injected before immunity is established. That a vascular lesion might not ultimately
result from frequently repeated small doses is not proven by these experiments, but as far as they go they tend to show that this is the case. The lack of results reported by Frothingham (11) on two rabbits as previously quoted, also gives strength to this view. The impression has also been gained that it is easier to produce the lesion in large rabbits. The differences in results are not, however, entirely explainable on the basis of dose, time, or weight of the animal, nor do individual differences in susceptibility to the toxin seem an adequate explanation.

Briefly summarized, the lesions observed consist of a fatty degeneration and necrosis of the smooth muscle of the media with subsequent crowding together of the elastic fibers into a relatively compact layer. There is also a degeneration of the elastic tissue in the media and the degenerated tissue becomes extensively calcified. In severe cases there is also a necrosis of the overlying intima and deep ulcers are formed. In certain rabbits there is a definite cellular hyperplasia of the intima which appears to be independent of medial degeneration. This will be discussed later. In none of the animals in which extensive necrosis and calcification were observed was there any inflammatory reaction in the media or hyperplasia of the overlying intima. In these cases, however, the overlying tissue was largely necrotic and sufficient time had perhaps not elapsed for reparative or compensatory processes to take place. It is quite possible that regeneration and compensatory intimal hyperplasia might ultimately occur over these medial lesions if the animals could be kept alive. The cracks mentioned as occurring both in the aorta and peripheral vessels are to a considerable extent unavoidably produced post mortem by handling the vessels. That some of these fractures, however, which extend deep into the media, existed during life is proved by the fact that in several cases small dissecting aneurysms were found in the aorta which had manifestly been produced by the entrance of blood through the rupture in the intima and inner media into the necrotic zone in the deeper media. In one case a definitely antemortem clot had formed in one of the cracks and protruded as a small thrombus into the lumen of the vessel.

It is to be noted that four of the fifteen rabbits which received diphtheria toxin alone (Table I) showed gross vascular lesions as
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well as four of the seventeen rabbits which received toxin and pituitrin (Table II). It will also be noted that no calcification was demonstrated in any of the animals receiving toxin alone, while three of the animals receiving pituitrin with the toxin showed widely distributed and marked calcification. Between the two series, however, there was little difference in the gross characteristics or distribution of the lesions. The vessels in Nos. 6, 10, and 14 were almost as stiff and brittle as those in Nos. 17, 27, and 30, yet in the first three no calcification could be demonstrated either with hematoxylin or the von Kossa stain, while in the last three calcification was marked and widely distributed. Definitely palpable stiffening and a brittleness of such a degree that the vessel cannot be opened without producing many cracks extending through the intima and deep into the media are present before calcification takes place, due apparently to a degeneration of the elastic tissue which occurs in these severe cases along with the muscular degeneration. The severity of the lesion in these rabbits is shown in Fig. 10. Why calcification should occur in the fatty degenerated medial tissue of the rabbits which have received pituitrin, and not in the rabbits which have not, is difficult to explain. The two series differ only in the presence or absence of calcification, and it is felt that no emphasis should be laid on the importance of pituitrin in the production of this change. Possibly in a larger series this difference would not be so apparent. In fact, as already noted, Klotz (1) has obtained small plaques of medial calcification, limited, however, to the first part of the aorta, with diphtheria toxin alone. No case with small focal lesions was found in this series, the gross lesions being either diffuse and pronounced or entirely lacking.

The resemblance of these vascular lesions to the adrenalin type of arteriosclerosis has already been mentioned, and Klotz has called attention to the similarity of the latter lesion to the Moncheberg type of arteriosclerosis occurring in the peripheral vessels of man. In this connection it would seem appropriate to call attention to the following facts: that the lesions which have been experimentally produced with staphylococcus (Saltykow (3)), typhoid, and streptococcus (Klotz (1)) have been of the proliferative type affecting primarily and mainly the intima of the aorta and that lesions of the
peripheral vessels, except for focal lesions with staphylococcus, have not resulted; that the lesions obtained with diphtheria toxin have been degenerative and medial, closely resembling the Moncheberg type, but unlike this type in man, have been confined to the beginning of the aorta (Klotz (1)), or have involved the entire aorta as well as to a considerable extent the peripheral vessels (present experiments); and that, on the other hand, the lesions which have been mainly associated, from clinical and postmortem observation, with infectious diseases in man have been the proliferative intimal type affecting the aorta, and an acute degeneration of the smaller arterioles in some of the organs, such as the kidneys. From such results as these here reported one is tempted to argue for an infectious or toxic origin for this peripheral type of arteriosclerosis in man, characterized by medial degeneration and calcification; and, from the results reported with staphylococcus, streptococcus, and typhoid bacillus, for a similar origin for the intimal plaques of proliferation and fatty degeneration in the aorta. Moreover, the fact that up to the present the injection of different varieties of bacteria or their toxins into rabbits has resulted in different types of arteriosclerosis at once suggests a specific etiology for these types in man. One seems, however, hardly justified in so applying these results, especially to the extent of the last conclusion. The intima of the rabbit's aorta is very thin, consisting practically of a single layer of endothelial cells lying on the internal elastic lamina of the media, and the facts above recorded may possibly be largely accounted for by anatomical structure of the vessels and strength, rather than specificity of toxin. A weaker toxin might produce proliferative rather than degenerative lesions; a stronger toxin might produce degeneration of more highly differentiated cells, as smooth muscle, before endothelial cells or fibroblasts. Since the vessel walls in these rabbits are evidently affected by toxin from the lumen rather than through the vasa vasorum we obtain in these animals, with a thin aortic intima, a medial degeneration of the aorta as well as of the peripheral vessels. If these theoretical explanations are correct we might expect to obtain with a proper strength of toxin in an animal with a thick aortic intima a proliferative intimal lesion here and a degenerative medial lesion in the smaller vessels; and in the rabbit one might hope to find a smaller dose of toxin which would produce a
proliferative intimal lesion in the aorta and a medial degeneration in the smaller vessels, which are without the dense elastic laminae of the aorta and probably more permeable. In one rabbit, No. 14, which received a relatively small dose of toxin, we find this condition present—intimal hyperplasia in the aorta (Fig. 3) and medial degeneration in the smaller vessels (Fig. 10)—thus giving a certain amount of experimental evidence for the idea that the difference in the type of lesion experimentally obtained with staphylococcus, streptococcus, and typhoid bacillus on the one hand, and diphtheria toxin on the other, are not due to specific action on a certain type of cell but to strength of toxin and anatomic structure.

Of possible interest in connection with these experiments is the work done by various authors on the effect of diphtheria toxin on adrenal function. Langlois (19), Luksch (20, 21), and Hannes (22) claimed that diphtheria toxin decreased adrenalin secretion, and Ehrmann (23) claimed that it increased it. Bonnamour and Thévenot (24) injected rabbits subcutaneously with diphtheria toxin and at the same time intravenously with adrenalin. They believe that the aortic degeneration resulting from the combined action of diphtheria toxin and adrenalin is more severe than from adrenalin alone, and that therefore the adrenalin secretion is increased. They injected no animals with diphtheria toxin alone. Tscheboksaroff (25) believes that the previous disagreement is explained by his results on dogs. He found that for 10 to 15 hours after the injection of diphtheria toxin the adrenalin secretion was increased, that in a second stage occurring 24 to 27 hours after injection the adrenalin secretion was normal, and in a third stage, 48 to 96 hours after injection, adrenalin disappeared from the blood. Abramow (26) from work on rabbits, guinea pigs, and immunized horses, concluded that under the influence of large doses of diphtheria toxin the adrenalin secretion ceased, with minimum lethal doses it decreased, and with sublethal doses and in immunization it increased. Some of these results, as well as the morphological resemblance between this and the adrenalin type of arteriosclerosis, suggest the possibility of a relationship between the two. The fact that the adrenals of these rabbits are enlarged seems no evidence of an increase in secretory function. The adrenals are also enlarged in rabbits injected with other bacteria, as in a series injected over long periods with colon bacilli by the author (27), in none of which were any lesions of the large vessels present. The enlargement of the adrenals is probably due to the storage in the cortex of fat, largely anisotropic, from the fatty degeneration produced by the toxin. If there is an increase in secretory function it is independent of this enlargement. It seems probable that the vascular lesion reported is due to the direct action of the diphtheria toxin on the vessel walls rather than through the medium of adrenalin. The latter possibility cannot, however, be absolutely dismissed.
It is not the purpose of this paper to recount the acute changes produced in various organs by diphtheria toxin, which have been previously carefully studied and described by others. It is desired, however, to mention briefly the changes in certain organs. The spleens of the arteriosclerotic rabbits which withstood the treatment longest are in marked contrast, as to size, with those of the rabbits which lived only a few days and particularly with those of rabbits, previously reported (27), which have been injected with colon bacilli. In older arteriosclerotic rabbits the organ is shrunken, being one-third to one-half the normal size; microscopically the Malpighian bodies are atrophied and there is a diffuse increase of connective tissue throughout the reticulum of the pulp. In the rabbits injected with colon bacilli the spleen is much enlarged; microscopically there is atrophy of the Malpighian bodies with a marked deposition of amyloid. In the rabbits injected with diphtheria toxin the arterioles in the spleen show no marked lesions, but that their endothelium is affected is evidenced by the fact that the lumen sometimes contains large numbers of flat, desquamated, endothelial cells.

Babes (12), experimenting on rabbits with diphtheria toxin, describes swelling, proliferation, and desquamation of the epithelium of the kidney and also of the endothelial cells of the blood vessels with the formation of hyaline masses in their lumina. Welch and Flexner (13), working on guinea pigs, rabbits, and kittens with diphtheria toxin, describe hyaline alteration of the glomerular capillaries and smaller arteries, especially in kittens. The hyaline completely filled the lumen of some capillaries. There was also fragmentation of nuclei in the glomeruli and tubular epithelium on a small scale. Frothingham (11) has described necroses of the walls of the larger vessels of the kidneys in rabbits, with fibrin deposits in the lumen and hemorrhage into the surrounding tissue. The glomeruli in cases showed partial necrosis of the capillaries of the tufts with fibrin formation in them.

The results in these rabbits confirm in the main the previous observations. In the gross the kidneys were of normal size or somewhat enlarged, they were frequently deep red, but sometimes pale and opaque. The minute dark red spots, characteristic of glomerular hemorrhages, were frequent. In a few of the more typical cases these glomerular hemorrhages, and also small opaque white spots were thickly scattered over the surface and on section were seen in the cortex. No differences were noted, either in the gross or micro-
scopically, between the kidneys of the rabbits which received toxin
only and those of the rabbits which received pituitrin with the toxin
unless it was that the hemorrhages were somewhat more numerous in
the gross in the latter series. Even in this regard, however, the dif-
ference was not striking. Microscopically both vascular and glom-
erular lesions were present in nearly all the rabbits, as shown in
Tables I and II, and in most cases were frequent and well marked.
The vascular lesions consisted of swelling and desquamation of the
endothelial cells of the arterioles and small veins with numerous
lateral thromboses in these vessels. The glomeruli show necroses of
the capillaries with hemorrhages into the tufts and capsular spaces
and the formation of fibrinous and hyaline masses (Fig. 14). Casts
and red blood corpuscles are numerous in the tubules. There is con-
siderable pyknosis and fragmentation of the nuclei, and in some cases
a swelling and apparently early cellular proliferation in the tuft.
Some of the glomeruli show rather marked collections of polymorpho-
nuclear leukocytes in and about the lesions in the tufts (Fig. 15). It
seems appropriate to call particular attention to the occurrence of
these focal glomerular lesions with cellular infiltration in the kidney,
produced by a soluble toxin circulating in the blood, as did Welch
and Flexner (14) in the case of the focal lesions in the liver. A few
of the older cases show an early diffuse interstitial proliferation of
connective tissue, apparently quite different from the focal scars
occurring as a spontaneous lesion in rabbits. The Sudan stain shows
that fatty degeneration was always present, but varying much in de-
gree and extent. In the less severe cases the convoluted tubules
only are affected; in the more severe cases the tubular epithelium is
affected throughout. Moderate fatty degeneration in the tufts is
frequent.

Though these lesions do not differ essentially from those previously
reported, it would seem that the similarity of the nephritis to
acute glomerulonephritis in man has not been sufficiently emphasized.
Ophüls (28) has called attention to the importance of vascular changes
in glomerulonephritis in man, and, in these rabbits, the primary lesion
and essential factor in the production of the glomerular change ap-
ppear to be a similar vascular damage and the result is a nephritis
closely resembling the acute and subacute glomerular types in man.
It is unfortunate that it has thus far been impossible to keep a rabbit alive for more than 28 days after the injection of the large dose of toxin which seems essential for the production of the vascular lesions here reported, since a study of the subsequent morphological changes both in the vessels and in the kidneys, as well as of the functional changes resulting, would be of much interest.

CONCLUSIONS.

There may be produced in rabbits by the intravenous injection of large doses of diphtheria toxin a vascular degeneration involving the entire aorta, the carotids to the base of the skull, the subclavians, and iliacs, and, for a varying distance distally, the brachials, femorals, and large abdominal vessels. The first part of the pulmonary artery is sometimes affected. The lesion is practically diffuse throughout the aorta and vessels mentioned, consisting of a fatty degeneration and necrosis of the smooth muscle in a wide zone of the media and a crowding together of the elastic fibers in the region affected, resulting in an irregular thinning of the vessel walls and many small aneurysmal pouchings. In rabbits which received pituitrin with the diphtheria toxin extensive calcification occurred throughout this degenerated zone, both in the aorta and other large vessels. It is believed, however, that the pituitrin is not essential to the calcification and that if it is of any importance it is because an extreme fatty degeneration is produced more quickly in the media of the vessels when it is administered simultaneously with the toxin.

Diphtheria toxin, given in large doses intravenously, produces in the kidneys of the rabbit a pronounced vascular and parenchymatous degeneration. The former consists of a swelling and desquamation of the endothelial cells of the arterioles and small veins with the formation of fibrinous thrombi, a necrosis and thrombosis of the capillaries of the tufts with hemorrhage and the formation of fibrinous and hyaline masses, and in some of the affected glomeruli considerable collections of polymorphonuclear leukocytes.

In conclusion I wish to acknowledge my indebtedness to Dr. Ophüls for his interest and advice throughout the experiments, and particularly for his examination of the kidneys.
BIBLIOGRAPHY.


EXPLANATION OF PLATES.

PLATE 14.

**Fig. 1.** Rabbit 30. The aorta and its branches. All the vessels shown were diffusely calcified with the exception of the abdominal branches of the aorta and the iliacs. In these calcification was only slightly apparent in the gross. The irregular dilatation of the upper portion of the aorta is shown ending sharply at about the level of the right renal artery. A shallow but well marked dilatation is seen on the convex surface of the descending portion of the arch just below the left subclavian artery; also a fusiform dilatation of the pulmonary artery lying just above the left auricle. The irregular thinning of the walls is apparent, particularly in the abdominal aorta and the carotids. The transverse cracks are apparent even in this external view, seen best in the abdominal aorta and right carotid.

**Fig. 2.** Rabbit 30. The same as Fig. 1, but with some of the vessels opened. Note the irregular dilatation of the aorta ending sharply at about the level of the right renal artery; also the circumscribed dilatation of the aorta just below the left subclavian, a similar dilatation on the posterior wall of the proximal portion of the right carotid, and the fusiform dilatation of the pulmonary artery (not opened) lying just above the left auricle. The stiffness of the vessels is evidenced by the crumpled appearance, best seen throughout the thoracic aorta, and their brittleness by the numerous transverse cracks, best shown in the photograph throughout the aorta, but also present in the other vessels.
PLATE 15.

Fig. 3. Rabbit 14. Arch of the aorta. A marked cellular thickening of the intima is seen, without apparent lesions in the media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

Fig. 4. Rabbit 27. Arch of the aorta. A wavy zone of marked calcification in the media is present, of fairly uniform width but varying in depth below the intima. There is considerable necrosis with lack of nuclear staining in the overlying intima and media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

Fig. 5. Rabbit 17. Thoracic aorta. There is a zone of marked calcification in the upper media with necrosis of the intima and overlying media. On the left, calcified spicules have broken through and project into the lumen. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

PLATE 16.

Fig. 6. Rabbit 30. Abdominal aorta. There is extensive calcification in the media, complete sloughing of the intima with ulcerations extending deeply into the calcified areas of the media, and a cellular thickening with round cell infiltration in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

Fig. 7. Rabbit 27. Abdominal aorta. The wall of the vessel is largely necrotic and extensively calcified, with the exception of a narrow zone next to the adventitia. In the center of the photograph the media is of about normal thickness, the intima has sloughed, and two zones of calcification are seen, one on the surface and one in the deep media, the intervening medial tissue being necrotic with little nuclear staining. At either side are deep ulcers extending almost through the media. There is hemorrhage in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

Fig. 8. Rabbit 27. Abdominal aorta. A shallow aneurysmal dilatation is present. Otherwise the condition is similar to that in Fig. 7. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

PLATE 17.

Fig. 9. Rabbit 30. Abdominal aorta. Extensive necrosis and calcification of the media with broad shallow ulcers. Note the marked round cell infiltration in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. \( \frac{1}{4} \), oc. 1.

Fig. 10. Rabbit 14. Cross-section of the carotid artery. Necrosis of the muscle is seen throughout a narrow zone of fairly uniform width in the upper media with crowding together of the elastic fibers into a compact layer. There is an irregular thinning of the wall with the formation of shallow pouchings.

Fig. 11. Rabbit 17. Cross-section of the femoral artery. There is a zone of calcification in the upper media and at the right a second area of calcification and softening in the deeper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. ¼, oc. 1.

Plate 18.

Fig. 12. Rabbit 27. Cross-section of the carotid artery showing calcified plates in the upper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. ¼, oc. 1.

Fig. 13. Rabbit 17. Cross-section of a branch of the renal artery in the pelvis of the kidney, showing small calcified areas in the upper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. ¼, oc. 1.

Plate 19.

Fig. 14. Rabbit 10. Kidney. Lesions are present in all the glomeruli, consisting of partial necrosis of the tufts with the formation of fibrinous and hyaline masses. Many of the tubules contain casts. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. ¼, oc. 1.

Fig. 15. Rabbit 27. High power of a typical kidney glomerulus, showing degeneration and necrosis of the tuft with hemorrhage and the formation of hyaline and fibrinous masses and a rich infiltration with polymorphonuclear leukocytes. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. ¼, oc. 1.
(Bailey: Arteriosclerosis and Glomerulonephritis.)
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