EFFECT OF HYPERTENSION ON VASCULAR AND OTHER LESIONS OF SERUM SICKNESS

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There is abundant experimental and clinical evidence to indicate that hypertension may induce the development of widely distributed vascular lesions. In some experimental situations,1-8 as well as in patients succumbing to malignant hypertension,9,10 such lesions have exhibited a similarity to the vascular alterations characteristic of polyarteritis nodosa in man. Indeed, as first suggested by Meyer in 1878,11 some have ascribed a significant role to hypertension in the pathogenesis of this vascular disorder, at least in certain instances.2,3,12 Although hypertension may be absent in patients with polyarteritis, nevertheless the relationship between the occurrence of lesions in the pulmonary arteries and the existence of pulmonary hypertension does appear direct.13 Similarly, a close resemblance between the lesions of drug sensitivity and serum sickness in man14,15 and the rabbit16-20 and polyarteritis has been commented upon by a number of investigators. However, that there are significant histologic differences between the vascular lesions resulting from hypertension and the injection of foreign protein has been recently emphasized by Campbell and Santos-Buch21 who compared the histologic effects of hypertension produced by unilateral perinephritis and hypersensitivity following the injection of horse serum in rabbits.

Because of the possible etiologic significance of hypertension in the development of the vascular lesions in polyarteritis as well as its effect on vascular lesions in general, it was considered worth while to explore the effect of the hypertensive state on the vascular lesions induced in rabbits by the administration of foreign protein. This report is concerned with the results of such a study dealing with the effect of hypertension on the acute lesions of hypersensitivity as well as those in the healed stage.

MATERIAL AND METHODS

Eighty-three adult white male and female rabbits, weighing approximately 2.5 kg., were utilized in the following experiments. Body weights were recorded prior to and at 2-week intervals during the experimental periods. Animals were maintained on a

Accepted for publication, July 7, 1961.

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stock laboratory ration and water ad libitum. Blood was obtained prior to sacrifice for determination of the blood urea nitrogen (BUN) by the Kjeldahl technique.

**Group I. Induction of Hypertension**

The induction of hypertension was attempted in 15 rabbits by the method of Page, Salmoiraghi and McCubbin except that both unilateral nephrectomy and cellophane enclosure of the contralateral kidney were performed in one stage. Ten exhibited an average elevation of blood pressure greater than 15 mm. of Hg throughout the experimental period and were considered as hypertensive. Hypertension was first noted 7 to 10 days following operation. Animals in this group were sacrificed 30 to 50 days (average 43 days) following the onset of hypertension or after operation.

**Group II. Production of Lesions of Acute Hypersensitivity**

Eighteen rabbits were given a single intravenous injection of 250 mg. per kg. of bovine serum albumin (BSA) (Armour & Company), which was traced-labeled with I\(^{131}\) (IBSA) according to the method of Talmage, Dixon, Bukantz and Dammin. Animals were bled 5 minutes after injection and every 1 to 3 days starting on the third day following injection. Total IBSA activity was determined in aliquots of sera and converted to per cent IBSA of the initial 5-minute sample. Twelve animals exhibited curves characteristic of immune elimination of antigen between 13 to 16 days following injection. These were sacrificed on the day of antigen elimination. The remainder of the group were empirically sacrificed on day 17.

**Group III. Healed Hypersensitivity**

Twelve rabbits were treated identically to those described in group II except that they were all sacrificed 28 days following injection of IBSA. Eight had demonstrated a curve indicative of immune elimination of antigen.

**Group IV. Effect of Hypertension on Lesions of Acute Hypersensitivity**

The induction of hypertension was attempted in 22 rabbits. All received an injection of IBSA as noted in group II 32 days after operation. Twelve remained hypertensive throughout the experimental period and exhibited immune elimination of antigen 13 to 16 days following injection and were sacrificed at corresponding periods. Five hypertensive rabbits failed to exhibit immune elimination of antigen and were sacrificed 17 days following IBSA injection. In the remaining 5, hypertension failed to develop or persist to the time of IBSA injection. Three of these exhibited curves characteristic of immune antigen elimination.

**Group V. Effect of Hypertension on Healed Lesions of Hypersensitivity**

Sixteen rabbits in which hypertension had been successfully induced for approximately 15 days received an injection of IBSA as noted in group IV. Ten exhibited immune elimination of antigen 13 to 16 days following injection. All animals were sacrificed 29 days following injection of IBSA except one which died 22 days following injection.

**Estimation of Blood Pressure**

Blood pressure determinations were performed by the ear capsule technique of Grant and Rothschild. All readings were obtained by the same individual on animals in the same isolated room. A control blood pressure was obtained for each rabbit by calculating the average of 3 consecutive daily determinations. Blood pressure was determined every other day for 10 days following operation and every week thereafter.

**Anatomic and Histologic Studies**

At the time of sacrifice or death of the animals (one of group V, vide supra), the heart was cleaned of pericardial fat and adventitious tissue, and weighed. Comparable
blocks of heart, lung, aorta, pancreas, spleen, kidney, gonad, skeletal muscle, stomach, small intestine, colon, thyroid and adrenal from all animals were fixed in 10 per cent formalin and processed and embedded in paraffin in the usual manner. Sections were stained with hematoxylin and eosin, and by the Verhoeff-van Gieson method in selected instances.

The severity of lesions encountered was subjectively graded 1 to 4+. An arbitrary index of the degree of the vascular alterations encountered in each animal was expressed as the quotient of the sum of the grades of severity of lesions encountered in each organ divided by the number of organs affected.

RESULTS

Two rabbits in group II (normotensive, acute hypersensitivity) and a similar number in group IV (hypertensive, acute hypersensitivity) that did not exhibit immune elimination of antigen were found to have lesions comparable to other members of group II (vide infra) when empirically sacrificed at 17 days. However, only those animals receiving IBSA, demonstrating characteristic curves of this phenomenon, were included in the final tabulation of results. This practice afforded a common "end point" for the comparison of results obtained in the various experimental groups receiving IBSA.

General Effect of Cellophane Perinephritis and Injections of Foreign Protein (IBSA)

No significant changes in body weight were observed in any of the experimental groups (Table I). Animals subjected to cellophane perinephritis and contralateral nephrectomy exhibited less weight gain during the experimental period than untreated, unoperated control rabbits during a comparable period of observation. This effect did not appear to be related to the successful induction of hypertension in these animals. Moderate but comparable elevations of BUN were noted in all animals subjected to operation as well as those that received injections of IBSA and were sacrificed at the time of its immune elimination (groups II and IV). The administration of IBSA failed to influence the blood pressure in either normotensive or hypertensive animals.

Comparison of Lesions in Hypertensive Rabbits and Those Receiving IBSA

Aside from cardiac hypertrophy, the lesions observed in animals with successfully induced hypertension (group I) were almost exclusively limited to arteries of distributing and smaller caliber. As indicated in Table II, these were most frequently observed in the gastrointestinal tract and liver, and only rarely in a branch of the coronary arteries (Fig. 1). The vascular alteration was characterized by either medial hypertrophy with moderate lumen narrowing or mural hyalinization. Necro-
**Table I**

OBSERVATIONS IN NORMOTENSIVE AND HYPERTENSIVE RABBITS WITH AND WITHOUT HYPERSONSITIVITY (BSA)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Weight (kg)</th>
<th>Blood pressure (mm. of Hg)</th>
<th>Duration of hypertension (days)</th>
<th>Days following BSA Injection</th>
<th>Antigen elim. (day)</th>
<th>BUN †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Begin</td>
<td>End</td>
<td>Difference</td>
<td>Begin</td>
<td>End</td>
<td>Difference</td>
</tr>
<tr>
<td>I</td>
<td>Hypertension</td>
<td>10</td>
<td>2.3</td>
<td>2.5</td>
<td>+0.2</td>
<td>82</td>
<td>114</td>
</tr>
<tr>
<td>II</td>
<td>BSA, acute, normotensive</td>
<td>12</td>
<td>3.0</td>
<td>3.0</td>
<td>0</td>
<td>84</td>
<td>81</td>
</tr>
<tr>
<td>III</td>
<td>BSA, healed, normotensive</td>
<td>8</td>
<td>2.9</td>
<td>2.9</td>
<td>0</td>
<td>74</td>
<td>78</td>
</tr>
<tr>
<td>IV</td>
<td>Hypertension and BSA, acute</td>
<td>12</td>
<td>3.0</td>
<td>3.0</td>
<td>0</td>
<td>82</td>
<td>128</td>
</tr>
<tr>
<td>V</td>
<td>Hypertension and BSA, healed</td>
<td>10</td>
<td>2.4</td>
<td>2.6</td>
<td>+0.2</td>
<td>84</td>
<td>113</td>
</tr>
</tbody>
</table>

* Average from onset of hypertension.
† Normal range, 10 to 20 mg. %.
‡ One died at 22 days.
### Table II

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypertension</th>
<th>BSA, acute normotensive</th>
<th>BSA, healed normotensive</th>
<th>Hypertension and BSA, acute</th>
<th>Hypertension and BSA, healed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>9 / 2</td>
<td>5 / 1</td>
<td>75 / 2 / 1</td>
<td>17 / 2 / 1</td>
</tr>
<tr>
<td>II</td>
<td>5 / 1</td>
<td>7 / 1</td>
<td>15 / 1</td>
<td>75 / 2 / 1</td>
<td>17 / 2 / 1</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>9 / 1</td>
<td>5 / 1</td>
<td>75 / 2 / 1</td>
<td>17 / 2 / 1</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>9 / 1</td>
<td>5 / 1</td>
<td>75 / 2 / 1</td>
<td>17 / 2 / 1</td>
</tr>
</tbody>
</table>

*Lesions represented as per cent incidence/grade of severity.
†Lesions, hyaline or proliferative, in only one instance of group I.*
tizing arteritis with fibrinoid change of the medial coat and a moderate heterophil and lymphocyte cellular infiltration in all vessel coats was noted in only one instance. Examination of animals subjected to peri-nephritis in which hypertension failed to develop did not disclose any significant vascular or other alteration.

On the other hand, 50 per cent of normotensive rabbits examined following the immune elimination of injected IBSA (group II) disclosed the presence of proliferative glomerulonephritis and a valvulitis (Fig. 2). The latter was characterized by endothelial swelling and hyperplasia, subendothelial edema and infiltration of the cardiac valves with varying numbers of inflammatory cells. A granulomatous reaction was observed in the white pulp of the spleen in only one animal of this group. Arteritis of medium and small arteries, principally the coronary and renal arteries, was also evident (Fig. 3). The vascular lesions varied in intensity in a manner similar to that noted above as occurring within the heart valves but, in addition, not infrequently exhibited fibrinoid necrosis. Except for the presence of mild valvulitis encountered in one animal, rabbits of group III examined two weeks following the immune elimination of antigen failed to reveal any significant vascular or other alteration.

Effect of Hypertension on Lesions of Acute and Healed Hypersensitivity

As noted in Table II, the administration of IBSA to hypertensive rabbits (group IV) resulted in a marked increase in the incidence of glomerulonephritis (Fig. 4), valvulitis (Fig. 5) and arteritis (Fig. 6) when these animals were examined at the time of immune elimination of antigen. In addition, a marked increase of vasculitis was observed in sites more frequently affected by uncomplicated hypertension, viz., gastrointestinal tract (Fig. 7) and liver. Also, arteritis was observed in the gonad, thyroid, and lung in a substantial number of these animals although such lesions were not observed in either hypertensive (group I) or hypersensitivity (group II) controls. Although there were no qualitative differences observed in the lesions of hypersensitivity noted in hypertensive animals as compared to similarly treated normotensive controls, the intensity of the nephritis, vascular lesions and arteritis was more severe as reflected by the results of the arbitrary grading technique employed in this study for the estimation of degree of alteration at these sites (Table II). This effect did not appear to be directly related to the degree of elevation of blood pressure encountered.

It is also apparent from Table II that the hypertensive state had no effect on the healing process in the lesions of hypersensitivity. Rabbits of this group (IV) as well as members which did not exhibit immune elimination of antigen displayed lesions (Figs. 8 and 9) qualitatively and quantitatively similar to those in the hypertensive controls (group I).
DISCUSSION

The results of this study have indicated that the hypertensive state increased the incidence as well as accentuated the vascular, valvular and glomerulonephritic lesions of acute serum sickness induced by the administration of BSA. This effect is also reflected by a comparison of our results concerning the general incidence of acute lesions in hypertensive BSA-treated rabbits and those appearing in the apparently normotensive BSA-treated rabbits reported by Germuth and Weigle and Dixon (Table III). Analysis indicates that except for a slightly lower incidence

<table>
<thead>
<tr>
<th>Investigator</th>
<th>No. of animals</th>
<th>Blood pressure</th>
<th>Glomerulonephritis (%)</th>
<th>Valvulitis (%)</th>
<th>Arteritis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germuth (^{39})</td>
<td>9</td>
<td>Normotensive</td>
<td>89</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>Weigle and Dixon (^{20})</td>
<td>19</td>
<td>Normotensive *</td>
<td>78</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>Fisher and Bark</td>
<td>12</td>
<td>Normotensive</td>
<td>50</td>
<td>50</td>
<td>41</td>
</tr>
<tr>
<td>Fisher and Bark</td>
<td>12</td>
<td>Hypertensive</td>
<td>75</td>
<td>75</td>
<td>83</td>
</tr>
</tbody>
</table>

* Presumably normotensive; no mention of blood pressure.

of glomerulonephritis in the normotensive BSA-treated animals, similar values were obtained for other lesions of acute hypersensitivity, and the latter, particularly arteritis, were increased in animals with hypertension. It appears worth while to note that the incidence of coronary arteritis was profoundly influenced by the hypertensive state (Table II). In addition, hypertensive rabbits receiving BSA frequently exhibited vascular lesions in the gastrointestinal tract, liver and such miscellaneous sites as the thyroid and gonad, a distribution unlike that of the lesions in acute hypersensitivity but similar to that observed in untreated hypertensive controls. This information raises the query whether hypersensitivity might not have augmented the lesions ascribed to hypertension. Although we cannot unequivocally exclude this possibility, certain considerations tend to militate against it. The lesions encountered in the hypertensive rabbits exhibiting hypersensitivity appeared more intense than those observed in normotensive rabbits treated with BSA. Nevertheless they were qualitatively similar to those of the latter group, being characterized by varying degrees of vascular necrosis and fibrinoid change with a conspicuous inflammatory component. On the other hand, the lesions observed in the hypertensive controls in this and previous studies in which
this experimental model was utilized, were, except on rare occasions, predominantly noninflammatory, being characterized by hyalinization of vascular coats or medial hypertrophy. It might be noted that our findings in this regard are in general agreement with those of Campbell and Santos-Buch who observed a similar difference in the distribution pattern and histologic appearance of the lesions produced by hypertension induced by perinephritis and serum sickness resulting from the administration of foreign protein. Further, these authors rarely noted the occurrence of a necrotizing vascular lesion after 22 days of hypertension. The duration of hypertension in the studies reported herein was 40 or more days. Since cardiac valvular lesions as well as glomerulonephritis have been found only in rabbits receiving BSA, one would be compelled to conclude that the vascular lesions observed in the gastrointestinal tract were pathogenetically different from those noted in other sites. On the other hand, as indicated above, the qualitative similarity, although not wholly corroborative, does lend some support to their pathogenetic similarity. Lastly, if hypersensitivity primarily affected the lesions of hypertension, which at the time of administration of BSA should have been histologically "healed," one might expect to observe some evidence of chronicity in the lesions. Foci of necrosis have been observed in "healed" lesions of polyarteritis nodosa in man; these have been considered strongly suggestive of such an event.

The mechanism responsible for the augmentation of the acute lesions of hypersensitivity in hypertensive rabbits is not clearly evident. There is some evidence to warrant the consideration that this effect is the result of the hypertension per se. No significant differences in the degree of nitrogen retention or change in body weight could be detected which might be construed to play a role in accounting for the divergent observations. More direct support for the primacy of the role of hypertension may be obtained from the animals exhibiting immune elimination of antigen, in which hypertension failed of induction or failed to persist throughout the experimental period. The incidence and severity of lesions in these were comparable to those encountered in normotensive BSA-treated rabbits. Since no significant fluctuations in blood pressure were noted in hypertensive or normotensive rabbits subjected to BSA injection, it seems highly unlikely that the effects observed might be due to blood pressure variations. In this regard, it is of interest that no direct relationship could be discerned between the degree of blood pressure elevation among the individual hypertensive BSA-treated rabbits and the severity of vascular lesions. Whether hypertension affects the nature of antigen-antibody complexes probably playing a role in the pathogenesis of serum sickness or renders vessels more susceptible to their action remains to be demonstrated. In regard to the latter, it is note-
worthy that hypertension clearly accentuates the development and severity of another vascular disease, notably cholesterol atherosclerosis, and that this effect may supersede such inhibitory or protective influences as diabetes or the administration of cortisone. The effect of hypertension in this regard has been considered attributable to its damaging effect on vessels.

Although the acute lesions of hypersensitivity were augmented by hypertension, this modality had no effect on the so-called healed lesions (group IV) in which vascular and other lesions were only exceptionally apparent in normotensive animals (group III). Qualitatively and quantitatively, the lesions in such animals resembled those encountered in the hypertensive controls. This information indicates that hypertension does not interfere with the healing process encountered in the vasculitis of hypersensitivity, despite the greater intensity of these lesions during their acute stage than observed in similarly treated normotensive rabbits. Moreover, hypertension was incapable of prolonging the duration of the lesions of hypersensitivity or producing vascular or other sequelae. This information also signifies the primacy of more prolonged antigenic stimulation or factors other than hypertension in the production of persistent vascular lesions considered to be immuno-allergic in nature. Although analogies between these experiments and the possible relationship of hypertension and polyarteritis or other forms of vasculitis in man do not appear warranted, it is well recognized that the lesions of polyarteritis nodosa may exhibit healing despite the presence of hypertension. Indeed, this characteristic represents one of the diagnostic criteria of this vascular disorder.

**Summary**

The successful induction of hypertension resulted in an increased incidence and severity of the renal, cardiac and vascular lesions, particularly those of the coronary arteries, in rabbits with serum sickness, as compared to normotensive controls. The hypertensive rabbits with serum sickness also displayed a high incidence of arteritis of the gastrointestinal tract, liver, and other sites more characteristic of the distribution resulting from uncomplicated hypertension. However, in the latter instance the vascular alterations were notably proliferative or hyaline rather than inflammatory as in the case of animals receiving foreign protein. This effect of the hypertensive state appears to be related to the hypertension *per se*. Its exact pathogenetic mechanism in this regard, however, remains to be elucidated. Attention is directed to the recognized damaging effect of hypertension on vessels, which may render the latter more susceptible to the effects of serum sickness.

On the other hand, hypertension failed to influence the resolution
of the lesions of serum sickness in rabbits sacrificed 1 to 2 weeks following the immune elimination of antigen. It is concluded that hypertension exerts little influence on the lesions of serum sickness, which in some respects resemble those of polyarteritis nodosa in man, except during their acute phase.

REFERENCE


The authors are grateful for the technical assistance of Maria Deichmiller in the preparation of IBSA.

[ Illustrations follow ]
Photomicrographs were prepared from sections stained with hematoxylin and eosin.

**Fig. 1.** A branch of the hepatic artery from a hypertensive control rabbit (group 1) exhibiting marked medial thickening. × 100.

**Fig. 2.** A representative degree of valvulitis in a normotensive rabbit which received an injection of bovine serum albumin (BSA). Endothelial proliferation, edema and scant inflammatory infiltrate are evident. The reaction is less severe than that noted in Figure 5. × 100.

**Fig. 3.** A mild degree of coronary arteritis in a normotensive rabbit receiving an injection of BSA. Compare with the alteration depicted in Figure 6. × 100.

**Fig. 4.** Proliferative glomerulonephritis of moderate degree in a wrapped kidney from a hypertensive rabbit that received BSA and was sacrificed at the time of immune elimination of antigen. × 100.
FIG. 5. Severe valvulitis is observed in a hypertensive rabbit receiving BSA (group IV) and sacrificed at the time of immune elimination of antigen. × 100.

FIG. 6. Severe coronary arteritis is observed in a hypertensive rabbit receiving BSA (group IV) and sacrificed at the time of immune elimination of antigen. × 100.

FIG. 7. Severe arteritis appears in the submucosal vessels of the stomach in a hypertensive rabbit receiving BSA and sacrificed at the time of immune elimination of antigen. × 100.

FIG. 8. A hyalinized submucosal artery in the small intestine of a hypertensive rabbit that received BSA but was sacrificed several weeks after immune elimination of antigen (group V). × 100.

FIG. 9. Medial hypertrophy is apparent in a branch of the coronary artery from a hypertensive rabbit sacrificed after immune elimination of antigen (group V). The lesion is qualitatively similar to that in the hypertensive control depicted in Figure 1. × 100.