In 1963 Okamoto and Aoki (1) had separated, by selective inbreeding, a colony of Wistar rat with 100% incidence of spontaneous hypertension. These rats were named spontaneously hypertensive rats (SH. rats) and, thereafter, various investigations of these SH. rats have been carried out by Okamoto et al. Although this SH. rat was distributed to many other laboratories in Japan as well as in other countries, the accurate longevity and mortal causes of the SH. rats remain still to be reported. Because of high incidence of pulmonary and other infections in the conventional circumstances, many rats died in early age accidentally. These interfered seriously with the studies on the sequences of developments of hypertension and its pathophysiological etiology. The present work revealed that the maintenance of the SH. rats in the specific pathogen free (SPF) condition prevented the animals from the infections and brought about the exact observation of the causes of the spontaneous death and of the hypertensive lesions at the advanced ages.

MATERIALS AND METHODS

Twenty-five male SH. rats and 14 male normotensive Wistar (N.) rats of SPF derivation were maintained in the following conditions for 20 months: The SH. rats of 13 and 14 generations and the N. rats were weaned from mothers at the age of 4 weeks. Five to 7 animals housed in a cage were kept at constant temperature and humidity. Lighting was regulated to give 13 hours of indirect illumination each day. The animals were allowed free access to drinking tap water and ordinary commercial diet (Niphon CLEA, CE-2) previously vapor-sterilized for 20 minutes. The diet was composed of natural foods containing approximately 24.0% of crude protein, 3.5% of crude fat and 56.0% of carbohydrate. All of the essential vitamins and minerals were present in amounts adequate for growth and maintenance, even after vapor-sterilization. The other environmental requirements, such as cages or bottles, for maintenance of the animals were sterilized with vapor or ethylene oxide periodically. The body weights of
the animals were measured at least once or twice a week. The general conditions of the animals were observed everyday.

The total cholesterol, crude fat, and phospholipid contents in the plasma and liver were measured in the 23 male SH. and N. rats, sacrificed at the ages of 5, 10, 25, 48, and 82 weeks, according to the methods of Abel et al. (2), Folch et al. (3) and King (4), respectively. The lipids in the large thrombi were also measured by the same methods. The systolic blood pressure and the levels of electrolytes in the serum as well as in the thrombi were measured following the methods previously reported (5, 6). Calcium content was measured by atomic absorption spectrometry with PERKIN Elmer Model 303. The animals died or sacrificed during the experimental period were subjected to autopsy for the macroscopical findings of the abnormal lesions. Some of the tissues were sectioned for the histological observations, the results of which will be described elsewhere.

RESULTS

1. Mortality and survival rates of the SH. rats

The SPF SH. rats as well as the conventional SH. rats showed a progressive elevation of the blood pressure with advancing age, but growth rate of the former was more marked than the latter (7). Eight and 13 out of the 25 SH. rats died at 17 and 20 months of age, respectively. However, among these rats 9 rats sacrificed at the pre-lethal state were included. Thirteen out of the 14 N. rats were healthy in appearance within 20 months of age. One N. rat showed progressive decrease in body weight at the age of 16 months and proved to have renal insufficiency at autopsy. The results are summarized in Fig. 1.

![Fig. 1. Mortality of the spontaneously hypertensive rat (SH. Rat) and normotensive Wistar rat (N. Rat).](image)

2. Gross autopsy findings in the SH. rats

As described before, 21 of 25 SH. rats died or were killed within 20 months of age and the remaining 4 SH. rats and 13 N. rats were also sacrificed at the age of 20 months.
As shown in Table 1, the macroscopical abnormalities detected in the SH. rats were mainly bead-like appearance of the mesenteric arteries with the thrombi in the vascular lumen, diffuse serosal haemorrhage and edema along the vessels, marked swelling of the heart, congested liver, sonorous pulmonary crackles, grossly enlarged kidneys, and ascites. These lesions were found in 20 SH. rats that died or were sacrificed within 20 months of age.

TABLE 1. Macroscopical hypertensive lesions observed in 20 SH. rats died or sacrificed within 20 months of age.

| Kind of lesion                  | Number of cases (cases) | Kind of lesion                  | Number of cases (cases) |
|---------------------------------|-------------------------|---------------------------------|-------------------------|
| Bead-like appearance            | 11/20                   | Systemic edema                  | 9/20                    |
| in mesenteric arteries          |                          | Thrombosis in hepatic veins     | 10/20                   |
| Nephrosclerosis                 | 17/20                   | Thrombosis in auricle           | 10/20                   |
| Cardiac hypertrophy             | 18/20                   | Hydrothorax                     | 6/20                    |
| Cerebral hemorrhage             | 1/20                    | Ascites                         | 2/20                    |

Fig. 2. A typical large thrombus (arrow) in the hepatic vein of a SH. rat. Several hemangiectasia-like lesions are observed in the congested liver.

Fig. 3. A medium- and three small-sized thrombi (arrows) in the hepatic vein of a SH. rat.
or sclerosis of the kidneys, cardiac hypertrophy with thin layered heart apex, systemic edema, and thrombosis in the hepatic veins (Fig. 2, 3) and left auricle (Fig. 4, 5). The details on these thrombi found in the hepatic vein and auricle are described afterwards. Accumulation of body fluid in the thoracic and peritoneal cavities was also observed in some of the SH rats with the thrombi in the auricle or hepatic veins. A localized hemorrhage in the ventral surface of the posterior hypothalamus was observed in one SH rat, who indicated a marked hypertension (over 220 mmHg) and the severe bead-like changes of the mesenteric arteries.

On the other hand, N. rats showed no macroscopical changes, except a slight degree of bead-like changes of the mesenteric arteries in 4 animals (29%). The thrombi were not observed in the N. rats.
Various sizes of thrombi whitish in color were found in the lumen of hepatic vein before entrance of the inferior vena cava, left auricle which was markedly dilated, or sometimes in the pulmonary vein of the edematous SH. rats. In one SH. rat a large thrombus was found in the cardiac cavity near the aortic valves. Some of the thrombi were occlusive in size, red in color, attached to the vascular wall, or organized.

In contrast to systolic blood pressure ranging from 190 to 220 mmHg in the hypertensive but non-edematous rats at age of 15 to 20 months, the edematous hypertensive rats at the same age showed the blood pressure ranging from 120 to 170 mmHg. Therefore, the development of the systemic edema served in some manner to lower the developed hypertension. The protein contents, measured by using a refractometer, in the thoracic and peritoneal fluid of the edematous hypertensive rats were 1.0 to 1.8 g/dL, and these values were only 1/3 to 1/5 of the protein content in the serum. However, the contents of sodium and potassium in the fluid were somewhat less than those in the serum.

3. Biochemical analysis of serum, liver and thrombus

The isolated thrombi did not dissolve with alcohol and chloroform, but became turbid by treatment with 1N HCl. The crude fat and total cholesterol contents of 4 thrombi were 3.85±0.50 (S.D.) g and 0.68±0.04 (S.D.) g per 100 g wet weight mass respectively, and the latter value indicated about 3 times of cholesterol in the liver. As shown in Table 2, the level of total plasma cholesterol in the SH. rats at the ages from 10 to 82 weeks was found to be low by 19 to 30% from that of the N. rats, about the younger rats (5 weeks) of both strains had not differences in their levels. On the other hand, the levels of the crude fat and total cholesterol in the liver were variable according to the ages. A slight but significant elevation of the total liver cholesterol in the SH. rats was observed at the age of 82 weeks. The levels of phospholipids in the serum and liver were slightly decreased in the SH. rats.

| Table 2. Cholesterol and phospholipids contents of plasma and liver in normotensive (N) and spontaneously hypertensive (SH) rats. |
|-----------------|---------|---------|---------|---------|---------|
| Age (weeks)     | 5       | 10      | 25      | 48      | 82      |
| Body weight (g) | N       | 109±11  | 329±24  | 428±30  | 467±28  | 492±40  |
|                 | SH      | 66±15*  | 252±18* | 393±13  | 401±18* | 376±26* |
| Blood pressure (mmHg) | N | 114±4 | 129±8 | 126±6 | 135±6 | 133±8 |
|                 | SH      | 119±4*  | 167±9*  | 207±17* | 200±9*  | 169±28* |
| Plasma total cholesterol (mg/100 ml) | N | 84±5 | 84±11 | 95±5 | 106±21 | 135±17 |
|                 | SH      | 83±4 | 60±14* | 72±6* | 83±11 | 110±9* |
| Plasma phospholipids (mg/100 ml) | N | – | – | 125±22 | 130±28 | 157±7 |
|                 | SH      | – | – | 93±16 | 119±9 | 130±48 |
| Liver total cholesterol (mg/100 g) | N | – | – | 232±10 | 211±10 | 234±12 |
|                 | SH      | – | – | 222±10 | 218±12 | 268±28* |
| Liver phospholipids (g/100 g) | N | – | – | 3.83±0.18 | 3.07±0.14 | 3.67±0.18 |
|                 | SH      | – | – | 3.41±0.21* | 2.86±0.16 | 3.45±0.30 |

* P<0.05. All values are mean and standard deviation of results in 4 or 5 different rats. Two edematous SH. rats are included among 4 SH. rats of 82 weeks of age. 100 g = wet weight.
The contents of water, sodium, potassium, and calcium of thrombi from edematous SH. rats were 76%, 526 and 56 meq/kg dry weight, and 61 mg/100 g wet weight respectively in the average of 3 samples. The potassium content in the thrombi was markedly lower than those of the vascular wall (110–140 meq/kg) and heart muscle (300–340 meq/kg). This reflected well with a little presence of the cellular component in the mass.

DISCUSSION

Since the exact observation on the longevity, mortal cause, and progression of the cardio-vascular lesions in the SH. rats was difficult in the maintenance of the animals under the conventional condition, the SH. and N. rats in this laboratory were kept in the SPF condition. Although the SPF condition prevented the infections and the resultant accidental death of the animals, the SH. rats were still short-lived. In contrast to death of one of 14 N. rats, 21 of 25 SH. rats died within 20 months of age. Berg et al. (8) showed that the mortality of the Sprague-Dowley rats 500 to 599 days in age, kept at the condition to prevent effectively the pulmonary infections, was 4 to 9% in male and 0 to 6% in female, and that the longevities of the male and female rats were 900 to 990 days and 1,100 to 1,199 days, respectively. The mortality of the conventional rats of Wistar-King Aptekman strain within 18 months of age was to be 32% in male and 24% in female (Usui, personal communication). Thus, the short lifespan of the SH. rats was confirmed even at the SPF condition.

The main causes of death in the SH. rats died within 20 months of age were not pulmonary infections but severe bead-like changes in the mesenteric arteries, hypertrophy of the heart, nephrosclerosis, and thrombosis associated with systemic edema, hydrothorax and ascites formation. These lesions accompanied with the malignant hypertension were detected by 90% in heart, 85% in kidney, 55% in mesenterium, and 45% as systemic edema. The appearance rate of the vascular lesions in the SPF SH. rats was much higher than that observed by Okamoto (9) in the conventional SH. rats. Moreover, the systemic edema as well as the presence of thrombosis in the hepatic veins and left auricle of the animals above 14 months of age were not observed by Okamoto et al. These differences may be due to the prolongation of lifespan or the more progressed generation of the SH. rats used in the present experiment.

The true causes of formation of thrombi favorable in the hepatic vein and left auricle are not clear, but it may be related to the hydrodynamic or rheologic problem in lumen of blood vessels and structural changes in the vascular walls. Preliminary observation using scanning electronmicroscope indicate that primary development of the thrombi may be concerned with deposition of thrombocytes on the walls of blood vessels. The thrombi in the hepatic vein presumably contribute to the elevation of the venous pressure and consequent increase of lymph, and the same events in the left auricle and pulmonary vein may contribute to cause the stagnation of blood in the lungs. Both events were much likely to activate the accumulation of the transudate fluid, which was low in protein content compared with the serum, in the body cavities.
The large thrombi proved markedly low in contents of cholesterol and calcium. This indicates that the thrombi are not formed with the conglomeration of either fat or calcium. The contents of water and sodium in the mass were higher than those observed in the vascular wall and heart (6), while the content of potassium was markedly low. Further, since the large thrombi in hepatic vein and auricle were very poor in the cellular component microscopically, it seemed to be composed of fibrin-like substance. The histological study of the tissues as well as the histochemical and electronmicroscopical studies on the thrombi are now in progress.

Renaud (10) has observed that the intravenous injection of gram-negative bacteria endotoxin induces the formation, in rats fed a hyperlipemic diet, of occlusive thrombosis primarily located in the large hepatic veins. Recently, Renaud and Godu (11) reported that the subcutaneous injection of epinephrine initiated the production of large thrombi in the left atrium of male rats fed a butter-rich diet for 10 weeks. Further, Renaud and Allard (12, 13) had reported that hyperlipemia seemed to be a necessary prerequisite for the induction of these thrombosis, but definite relationship could not be found between the tendency to thrombosis and the level of cholesterol, triglycerides and glucose in the serum and vitamins in diet. However, the SH. rats with the thrombosis did not merely indicate hyperlipemia, but indicated rather hypolipemia. Therefore, hyperlipemia is not likely to be a necessary factor for the spontaneous formation of the thrombi in the SH. rats.

The SH. rats, once the systemic edema developed, showed somewhat lower level of the blood pressure than the SH. rats without the edema, and showed a significant decrease in the motor activity and some respiratory dyspnoea seemingly due to hydrothraux. Histologically, some degenerative signs of the liver (unpublished work) were also observed. Therefore, the decrease in the level of blood pressure after development of systemic edema may derive mainly from the circulatory disorders or partially from some hypotensive factors in the damaged liver (14, 15).

SUMMARY

Longevity and the hypertensive lesions of the male spontaneously hypertensive (SH.) rats were observed under the specific pathogen free condition. Longevity of the SH. rats was markedly shorter than that of the control Wistar rats, and the mortality at 20 months of age was 84% in the SH. rats and 7% in the control rats. The macroscopical hypertensive lesions detected in the SH. rats were mainly bead-like appearance of the mesenteric arteries (55%), cardiac hypertrophy (90%), nephrosclerosis (85%), thrombosis accompanied with systemic edema (50%) and cerebral hemorrhage (5%). The formation of the occlusive thrombosis in the SH. rats primely located in the large hepatic veins as well as the left auricle, and did neither correlate to the hypercholesteremia nor hyperlipemia.
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