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Acute, massive, haemorrhagic adrenal necrosis experimentally produced by the Shwartzman mechanism in rabbits*

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Summary. Acute and severe haemorrhagic necrosis of the adrenal was produced experimentally in rabbits by means of intravenous injection of endotoxin after pretreatment by adrenocorticotropic hormone (ACTH) administration. The change occurred mainly in the *zona fasciculata* of the adrenal cortex, and its pathology was quite similar to that of the Shwartzman reaction. Numerous microthrombi were found in and around the lesion, but no marked changes were seen in other parts of the body. Heparin administration was very effective in preventing the necrosis. The pathogenesis of this lesion was postulated to be a univisceral Shwartzman mechanism in the adrenal. This seems to be a good experimental model for massive haemorrhagic necrosis of the adrenal in man, for example in the Waterhouse-Friderichsen syndrome, the pathogenesis of which has been assumed to involve intravascular clotting. It is suggested that hyperfunction of the adrenal cortex caused by ACTH administration could be a preparative condition for the Shwartzman reaction.

Key words: Adrenal haemorrhagic necrosis – Univisceral Shwartzman reaction – Waterhouse-Friderichsen syndrome – Preparative condition of Shwartzman reaction – Disseminated intravascular coagulation

Introduction

Acute, massive, and severe haemorrhagic necrosis of the adrenal, so-called adrenal apoplexy, is one of the most common causes of death due to acute adrenal insufficiency. A good example is the Waterhouse-Friderichsen syndrome (Friderichsen 1918; Waterhouse 1911), which occurs mainly in children, being accompanied by meningococcal, other bacterial or viral infections, and which takes a fulminant course in most cases.

The pathogenesis of this lesion has long been studied and hypotheses have been advanced by many researchers including Berte (1953) and Becker et al. (1967), both of whom stressed the significance of infection and thrombosis. However the so-called generalized Shwartzman reaction in experimental pathology, or disseminated intravascular coagulation (DIC) in clinical medicine has been suggested to be involved in the pathogenesis, from both clinical and pathological standpoints (Fox 1971; Hjort and Rapaport 1965; Margaretten and McAdams 1958), though the relation is still controversial (Böhm 1982). For example, the predisposing factors for such acute and severe adrenal necrosis are thought to be quite similar to those for DIC, namely, endotoxaemia, pregnancy, the tumour-bearing state and others, and many case reports have appeared along this line (Hoffmann 1977; Young and Sweeney 1977).

Experimentally, diffuse haemorrhagic necrosis of the adrenal has been produced in animals by intravenous injection of endotoxin after stimulation by adrenocorticotropic hormone (ACTH) (Bavaud 1973; Haller 1969; Levin and Cluff 1965; Margaretten et al. 1965). According to Levin and Cluff (1965) however, such a phenomenon (as well as the adrenal changes in Waterhouse-Friderichsen syndrome) could not be considered to be a Shwartzman reaction because thrombi were not present in the lesion and because the change was not suppressed by heparin administration.

Through routine studies of human autopsy cases, we have obtained pathological findings suggesting the participation of the Shwartzman reac-

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tion in adrenal haemorrhagic necrosis. Therefore we repeated Levin and Cluff's experiment (1965) and rescrutinized their interpretation of their results on the basis of our own data. In this paper, we present the results of our experimental study, and discuss their significance in relation to the pathogenesis of acute, diffuse, haemorrhagic necrosis of the adrenal in man and animals.

Materials and methods

Japanese albino adult male rabbits weighing 3.5–4.0 kg were used. Experimental procedures are summarized in Table 1.

First, ACTH (tetracosactide acetate, durable synthetic ACTH preparation, Cortrosyn Z, Organon Manufacturing Co. and Daiichi Seiyaku Co., Tokyo, Japan) was injected intramuscularly into the animals 3 times (40 units each time) at intervals of 24 h, to stimulate the function of the adrenal cortex. Rabbits of experimental groups I and II were injected intravenously with endotoxin (*E. coli* endotoxin, 0111:B4, DIFCO Laboratories, Detroit, Michigan, USA) *via* the ear vein 24 h after the third injection of ACTH. The dose of endotoxin was 0.05 mg in group I and 0.1 mg in group II per 1 kg of body weight. Twenty-four hours after the endotoxin injection, the rabbits were sacrificed by intravenous, overdose administration of sodium pentobarbital (Somnopentyl, Pitman-Moore Manufacturing Co., Washington Crossing, NJ, USA), and autopsied. Rabbits that died within 24 h after the endotoxin injection were also autopsied as soon as possible after death.

Table 1. Experimental procedures

	0	1	2	3	4 days
Group I	ACTH 40 U (im)	ACTH 40 U (im)	ACTH 40 U (im)	Ex 0.05 mg/kg (iv)	autopsy
Group II	ACTH 40 U (im)	ACTH 40 U (im)	ACTH 40 U (im)	Ex 0.1 mg/kg (iv)	autopsy
Group III	ACTH 40 U (im)	ACTH 40 U (im)	ACTH 40 U (im)	Hp5000 U Ex 0.1 mg/kg (iv)	autopsy
Group IV	ACTH 40 U (im)	ACTH 40 U (im)	ACTH 40 U (im)	Sa 0.5 ml (iv)	autopsy
Group V	Sa 2 ml (im)	Sa 2 ml (im)	Sa 2 ml (im)	Ex 0.1 mg/kg (iv)	autopsy

Abbreviations: ACTH: adrenocorticotropic hormone; Ex: endotoxin; Hp: heparin; Sa: saline; im: intramuscular injection; iv: intravenous injection

Both adrenals were cut longitudinally and observed precisely. The adrenals, brain, hypophysis, heart, lungs, spleen, liver, pancreas, and kidneys were fixed in formalin. They were embedded in paraffin, thin-sectioned, and stained with haematoxylin-eosin (HE) and phosphotungstic acid-haematoxylin (PTAH) for histological examinations.

In the animals belonging to group III, adrenal cortical hyperfunction was produced by the same method as in the other groups, but later heparin was administered almost simultaneously with the endotoxin injection. An intravenous injection of 1000 units of heparin was given just before the endotoxin injection, and another 4000 units were injected subcutaneously 1 h and 15 min later. In the rabbits of group IV, saline, instead of endotoxin, was injected intravenously after ACTH stimulation. In group V, saline was injected intramuscularly instead of ACTH, followed by provocation with endotoxin. These three groups (III–V) were served as controls; the animals were examined pathologically in the same manner as the experimental animals.

Results

The results are summarized in Table 2. In groups I and II, all the rabbits showed necrosis of both adrenal glands to a greater or lesser degree. There was no significant difference in results between group I and II, thus the outcome did not depend on the dose of endotoxin used. However, adrenal necrosis occurred in only a few rabbits of groups III and IV, and in none of group V. The grade of adrenal necrosis in groups III and IV, when produced, was much milder than that in groups I and II. Thus, it is clear that severe adrenal necrosis occurred almost exclusively in the animals which received both ACTH stimulation and endotoxin provocation, and that it was apparently inhibited by heparin administration.

The gross picture of the adrenal necrosis, as shown in Fig. 1, involved a relatively sharp border, irregular shape, and varying size. In milder cases there were many scattered miliary-sized spots of necrosis. Larger and confluent areas of necrosis with an irregular or map-like shape were seen in more severe cases. The necrosis usually appeared to originate in the adrenal cortex. In almost all cases, haemorrhage was seen in and around the necrotic area, and as a result, the adrenal became swollen.

The adrenal necrosis varied very much from case to case, from micro-necrosis that could only

Table 2. Results of experiments

Rabbits			ACTH (U) (im)	Ex (mg/kg) (iv)	Hp (U) (iv+ic)	Survival Time	Grade of adrenal haemorrhage and necrosis
Group	No	body weight					
I	A-25	3.8 kg	40×3	0.05		<2 h	+++
I	A-26	3.6 kg	40×3	0.05		24 h	++
I	A-27	3.7 kg	40×3	0.05		<24 h	++
I	A-28	4.0 kg	40×3	0.05		24 h	+
II	A-29	3.7 kg	40×3	0.1		24 h	++
II	A-30	3.9 kg	40×3	0.1		24 h	++
II	A-31	3.6 kg	40×3	0.1		<24 h	+++
II	A-32	3.5 kg	40×3	0.1		<24 h	+++
II	A-33	3.7 kg	40×3	0.1		24 h	+
III	A-34	3.6 kg	40×3	0.1	5000	24 h	−
III	A-35	3.9 kg	40×3	0.1	5000	24 h	−
III	A-36	3.6 kg	40×3	0.1	5000	24 h	−
III	A-37	3.7 kg	40×3	0.1	5000	24 h	−
III	A-38	3.6 kg	40×3	0.1	5000	<4 h	+
III	A-39	3.5 kg	40×3	0.1	5000	24 h	++
IV	A-40	3.6 kg	40×3			24 h	+
IV	A-41	3.6 kg	40×3			24 h	−
IV	A-42	3.5 kg	40×3			24 h	−
IV	A-43	3.7 kg	40×3			24 h	−
IV	A-44	3.8 kg	40×3			24 h	−
V	A-45	3.8 kg		0.1		24 h	−
V	A-46	3.6 kg		0.1		24 h	−
V	A-47	3.9 kg		0.1		24 h	−
V	A-48	3.5 kg		0.1		24 h	−
V	A-49	3.6 kg		0.1		24 h	−

Abbreviations: ACTH: adrenocorticotropic hormone; Ex: endotoxin; Hp: heparin; im: intramuscular injection; iv: intravenous injection; ic: intracutaneous injection; +++: massive haemorrhagic necrosis involving almost the entire adrenal; ++: moderate necrosis that was definitely observable microscopically; +: mild necrosis that could only be detected microscopically; −: no haemorrhagic necrosis

be observed microscopically to diffuse and severe necrosis that involved almost the entire adrenal. The severity of the necrosis was therefore classified as shown in Table 2, according to its spread estimated on the central cut surface of the adrenal. In all instances, adrenal necrosis, if it occurred, was observed bilaterally. In cases where the grade of necrosis differed from left to right, the more severe grade was taken for scoring. Of 9 rabbits belonging to groups I and II, 4 died within 24 h after the administration of endotoxin and all of these animals showed diffuse adrenal necrosis.

Histological findings of the lesion were fundamentally the same in all cases, regardless of the severity of necrosis. The border of the necrotic area was clear. Necrosis was observed mainly in the adrenal cortex, especially in the *zona fasciculata*, but the *zona glomerulosa* was sometimes involved as well. In the severest cases, necrotic lesion spread out from the cortex, and the medulla was also involved, with almost no normal adrenal tissue remaining. The character of the necrosis was mainly coagulative, being accompanied by leukocytic infiltration and haemorrhage, though lytic necrosis was also found rarely in a few cases (Figs. 2–4).

Small, fresh fibrin thrombi were found scattered in and around the necrotic areas in many cases, as shown in Fig. 5. Such microthrombi were most frequently observed in the sinusoids and small blood vessels, especially in the area adjacent to the necrosis. The number and distribution of microthrombi varied from case to case. Although no definite relationship between the number, or the mode of distribution of microthrombi and the severity or spread of the necrotic lesions was apparent, the main mechanism of the adrenal necrosis induced in this experiment was considered to be circulatory disturbance caused by microthrombus formation. Some thrombus formation was observed in non-necrotic parts of the adrenal in some cases and densely packed accumulations of red blood cells (haemagglutination) were seen quite often in the sinusoids as well as in the small blood vessels.

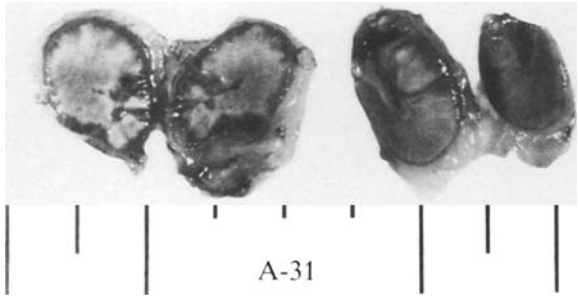


Fig. 1. Maximum cut surface of the swollen adrenals of group II rabbit with diffuse haemorrhage and necrosis involving the entire adrenals. Haemorrhage is especially severe in the adrenal cortex

In other organs than the adrenal, necrotic and/or haemorrhagic lesions of mild degree were seen infrequently in limited areas of the lung, liver, and spleen, but neither marked necrosis nor microthrombus was found anywhere.

Discussion

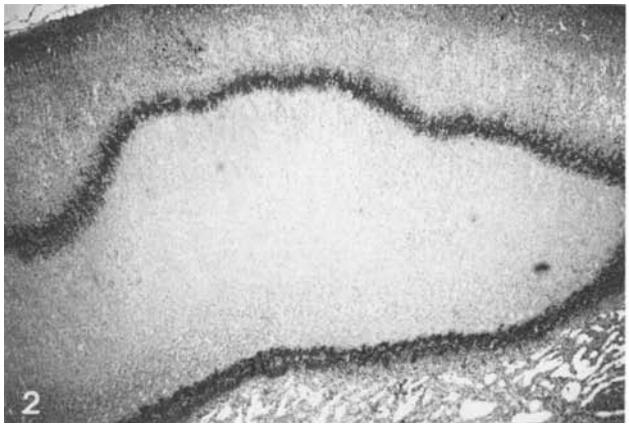
The localized Shwartzman reaction was found by Shwartzman himself in 1928 (Shwartzman 1928). Sanarelli's phenomenon (Sanarelli 1924) was found and reported 4 years prior to that, and is now called the generalized Shwartzman reaction, following the proposal of Apitz (1935). Many studies have been made on these reactions and at present it is thought that the fundamental mechanism of localized and generalized Shwartzman reactions is essentially the same, involving microcirculatory disturbances represented morphologically by microthrombi formation. The process is suppressed by heparin (Good and Thomas 1953) and warfarin (Shapiro and McKay 1958). Nowadays, it is widely accepted that the generalized Shwartzman reaction of experimental animals corresponds to DIC in human cases.

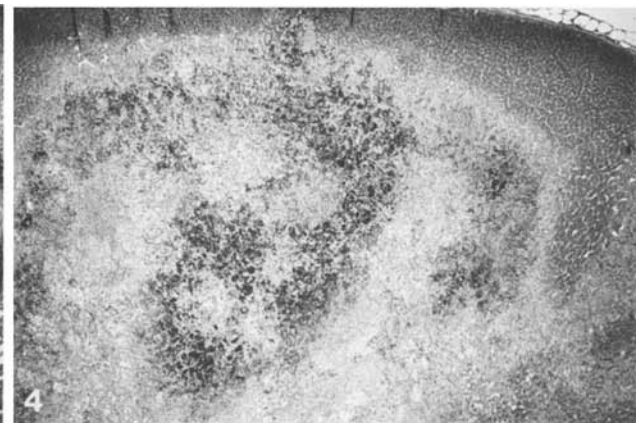
Recently, the univisceral or single organ type has been proposed as the third type of the Shwartzman reaction (Mori 1981). This new entity is not fundamentally different from the other two classical types in pathogenesis, but is different from them in the following three respects: firstly, the reaction occurs almost exclusively in the target organ with minimal or rare changes in the other parts of the body; secondly, within the target site the change is extensive; and thirdly, as a rule the involved viscus becomes severely disordered in function, causing a "disease" and often resulting in the host's death. It is thought that there are some human diseases for which this univisceral Shwartzman reaction could be a useful model for elucidation of the pathogenesis. Attempts to produce the univisceral Shwartzman reaction have succeeded in various organs including the liver (Mori et al. 1979; Mori et al. 1981), pancreas (Thal and Brackney 1954), gall bladder (Mori et al.: Acute, haemorrhagic-necrotic cholecystitis experimentally produced by the Shwartzman mechanism in rabbits. In preparation), intestine (Berry and Fraser 1968; Loeschke 1980), lung (Shiga and Mori 1985), and renal papilla (Nakano et al. 1984).

In our experiment, severe and massive haemorrhagic necrosis was successfully produced in the adrenal of rabbits. Many microthrombi were seen histologically in and around the lesion, and the reaction was inhibited by heparin administration, in contrast to Levin and Cluff's findings (1965). Since endotoxin was used to provoke haemorrhagic necrosis, since the changes that occurred were very similar to those of the Shwartzman reaction morphologically, since heparin very effectively prevented the lesion, and since there were no marked changes in other organs, we believe that the haemorrhagic necrosis produced in the present experiment represents a univisceral Shwartzman reaction in the adrenal. This could be a good model for adrenal apoplexy in man, since there are many similarities between the two from both clinical and pathological viewpoints. We postulate that human adrenal necrosis could also be produced by the Shwartzman mechanism.

In principle, two procedures are necessary to cause the Shwartzman reaction, preparation and provocation. In contrast to immune reactions, exact identity is not required between the two agents used in the procedures. Further, pregnancy (Berghaus and Ehry 1972), malignant tumours (Mori et al. 1986), and certain drugs such as steroid hormones (Latour et al. 1971) are known to produce a preparative condition in the host, and a provocative procedure would be enough to cause the generalized Shwartzman reaction. It is also worth mentioning here that steroid hormones or ACTH can suppress the reaction when administered immediately prior to the provocative procedure (Latour et al. 1972). In any case, such a systemic preparative effect of ACTH can be ruled out in the present experiments, since the reaction occurred only in the adrenal.

As far as we know, only a few reports have indicated that vigorously metabolizing tissues such as malignant neoplasms (Walker and Handman 1939) and placenta (McKay and Wong 1963) readily become targets of the Shwartzman reaction and are in the Shwartzman-preparative state. In the present experiment, adrenal cortical hyperfunction





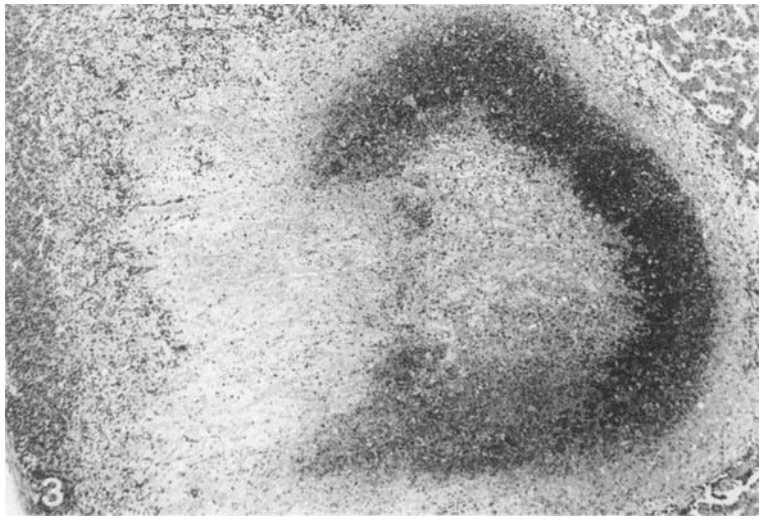


Fig. 2. Histological findings of relatively sharply demarcated necrotic lesions that are observed diffusely in the adrenal cortex of group I rabbit. Leukocyte-mononuclear cell infiltration in the circumference of necrotic area. Partially normal adrenal tissue can be observed in the subcapsular *zona glomerulosa* of the adrenal cortex. (HE stain, ×15)

Fig. 3. Necrotic lesion in the *zona fasciculata* of the adrenal cortex of group I rabbit. Marked infiltration and accumulation of leukocytes. (HE stain, ×35)

Fig. 4. Necrotic lesion with haemorrhage in the *zona fasciculata* of the adrenal cortex of group II rabbit. (HE stain, ×15)

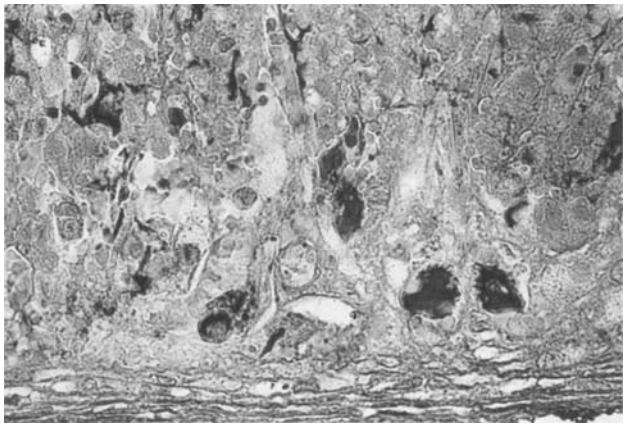


Fig. 5. High power view of the adrenal cortex of group II rabbit, showing small new fibrin thrombi in the sinusoids and small blood vessels of the subcapsular necrotic lesion. (PTAH stain, ×300)

caused by administration of ACTH is considered to have behaved as a preparative condition for the univisceral Shwartzman reaction of the adrenal. Thus, another example of hyperactivity, hyperfunction caused by hormone administration, is now shown to have the potential to be preparative.

The correlation between ACTH and adrenal injury, and further, that between stress and adrenal injury, may also be important. As regards ACTH, several reports have suggested that ACTH itself may give rise to adrenal injuries. There are some case reports (Galin 1958; Moolten 1957; Proctor and Rawson 1951; Redman and Faas 1976; Wilson and Roth 1953) on adrenal haemorrhage and necrosis which occurred suddenly in patients under ACTH administration.

Further, it is known that stress has a considerable influence on the occurrence of adrenal injuries and it has been suggested (Greendyke 1965) that stress of long duration could induce adrenal haemorrhage and necrosis. Since stress induces adrenal over-function through ACTH secretion, it has been claimed (Rich 1944) that stress may injure the adrenal tissue directly, or that a shock state caused by successive stress situations may provoke peripheral circulatory disturbance which would influence the adrenal. However, it is also a fact that diffuse haemorrhage and necrosis of the adrenal do not occur easily either after ACTH administration or under stress alone in men. Another, stronger stim-

ulation may be necessary to provoke the real lesion in the adrenal, and this could be infection or endotoxaemia.

We consider, on the basis of our results, that the univisceral Shwartzman mechanism may play an important role in the pathogenesis of adrenal haemorrhage and necrosis in humans. The present experiments are also considered to be significant in relation to the pathogenesis of Waterhouse-Friderichsen syndrome.

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