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ELICITATION OF THE GENERALIZED SHWARTZMAN REACTION IN RABBITS WITH PARENTERAL IRON PREPARATIONS AND BACTERIAL ENDOTOXIN

By

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ERIKSSON, HANS and GÖRAN MAGNUSSON: *Elicitation of the generalized Shwartzman reaction in rabbits with parenteral iron preparations and bacterial endotoxin.* Acta vet. scand. 1975, *16*, 226—235. — Some parenteral iron preparations were examined regarding their ability to act as preparing agents in the elicitation of the generalized Shwartzman reaction in the rabbit. As expression of the generalized Shwartzman reaction bilateral cortical necrosis of the kidneys was demonstrated when Imferon, Iroject, Macofer, and A 2152 were used as preparing agents. No renal lesion was observed when Jectofer was used.

generalized Shwartzman reaction; parenteral iron preparations; rabbit.

The generalized Shwartzman reaction characterized by bilateral cortical necrosis of the kidneys is well-known from experiments in laboratory rabbits. Under natural conditions the generalized Shwartzman reaction is rare, but attention has been paid to its significance (*Hjort & Rapaport* 1965). Spontaneous bilateral cortical renal necrosis interpreted as expression of a generalized Shwartzman reaction has been observed in man (*Krecke* 1964, *Marcussen & Asnaes* 1972), in the horse, cow, pig, dog (*Nordstoga* 1967, *Nordstoga et al.* 1968), and cat (*Hall* 1972).

The generalized Shwartzman reaction in the rabbit is classically induced by two appropriately spaced intravenous injections of bacterial endotoxin. The cortical renal necrosis is due

to fibrin thrombi formed mainly in the glomerular capillaries. The mechanism by which the first injection (the preparing injection) alters the response of the animal to the second injection (the provoking injection) is still unclear. It is known that elicitation of the generalized Shwartzman reaction is influenced by several factors. It is for instance prevented by heparin or a coumarin anticoagulant (*Good & Thomas* 1953, *Shapiro & McKay* 1958), by granulocytopenia induced by nitrogen mustard (*Thomas & Good* 1952 b, *Hjort et al.* 1964), by activation of the fibrinolytic system of the rabbit with streptokinase (*Condie et al.* 1957, *Kliman & McKay* 1958), and by α-adrenergic blocking agents (*Müller-Berghaus & McKay* 1967).

The susceptibility to the generalized Shwartzman reaction varies among the species (*Taichman* 1971). It is readily elicited in the rabbit in contrast to the rat which is considered resistant to the reaction. The susceptibility is increased under some conditions. The generalized Shwartzman reaction is easier to induce in immature rabbits than in adults (*Smith & Thomas* 1954). In rabbits and also in rats pregnancy prepares the animals and the Shwartzman reaction may be elicited by one single injection of endotoxin (*Apitz* 1934, *Wong* 1962, *McKay* 1963, *McKay & Wong* 1963). Adrenal steroids enhance the sensivity and may cause rabbits to develop the generalized Shwartzman reaction after one single injection of endotoxin (*Thomas & Good* 1952 a, *Latour et al.* 1971, *Collins et al.* 1972). A "blockade" of the reticuloendothelial system by Thorotrast or trypan blue (*Good & Thomas*) or colloidal iron in the form of saccharate of iron oxide (*Smith et al.* 1953, *Zweifach et al.* 1957) can replace the preparing injection.

As parenteral iron preparations are used in both human and veterinary medicine it was considered of interest to elucidate whether also other parenteral iron preparations may act as preparing agents in elicitation of the generalized Shwartzman reaction.

MATERIALS AND METHODS

The following parenteral iron preparations were used as preparing agents:

1. Imferon® (Pharmacia, Sweden): A high-molecular complex of iron and dextran.
2. Macofer® (Hausmann Laboratories, Switzerland): A high-molecular colloidal saccharate of iron oxide.

3. Iroject® (Astra-Ewos, Sweden): A high-molecular complex of iron, dextrin, sorbitol, citric acid and lactic acid.
4. Jectofer® (Astra, Sweden): A low-molecular complex of iron, dextrin, sorbitol and citric acid.
5. A 2152 (Astra, Sweden): A high-molecular complex of iron and a sorbitol-gluconic acid polymer.

The classification of parenteral iron preparations into high-molecular and low-molecular compounds is arbitrary. The molecular weight of iron-dextran and saccharated complexes of iron is reported to be larger than 150,000, while Jectofer, which is regarded as a low molecular compound, has a molecular weight of approx. 5000 (*Müller* 1974).

The iron concentration of Macofer was 20 mg per ml, that of Imferon, Jectofer and A 2152 was 50 mg per ml, and that of Iroject was 100 mg per ml. All preparations were administered undiluted intravenously.

The endotoxin lipopolysaccharide W from E. coli 0127:B8 (Difco Labs, USA) was used as provoking agent. A stock solution containing 1 mg endotoxin per ml saline was prepared immediately before injection.

This investigation included three experiments with a total number of 172 young white male and female rabbits weighing 0.5—0.8 kg. Groups of rabbits were given an intravenous injection (marginal vein of the ear) of an iron preparation. Endotoxin was injected intravenously 6 or in a few cases 3 hrs. later. Animals receiving parenteral iron or endotoxin alone were included as controls. Experiment A had a preliminary and tentative design to find out suitable dose levels for further studies (Table 1). In experiments B and C groups of 10 animals (five of each sex) were given the test compounds according to the schedules in Tables 2 and 3. The animals in experiment A were of conventional type while those in experiments B and C were of SPF-type.

The animals were killed by intravenous injection of sodium pentobarbitone 24 hrs. after the administration of the provoking agent. For the determination of the generalized Shwartzman reaction the kidneys were examined for cortical necrosis. Tissue samples of the kidneys and in experiment C also of the liver were preserved in 10 % neutral formalin solution. Paraffin embedded sections were prepared and stained with haematoxylin and eosin and according to Perl for iron. Frozen sections were

stained with haemalum and Sudan III for detection of fat. Some sections were stained according to Lendrum's acid picro-Mallory method.

RESULTS

Some rabbits died after administration of the provoking agent, namely 13 animals in experiment A, 14 in experiment B and six in experiment C (Tables 1, 2 and 3).

Table 1. *Experiment A:* Incidence of bilateral cortical necrosis in rabbits after intravenous injection of endotoxin following intravenous administration of endotoxin or some parenteral iron preparations.

Group	Treatment				Time between injections in hours	Number of animals	Mortality	Incidence of bilateral necrosis
	preparing agent	dose (mg/kg)	provoking agent	dose (mg/kg)				
1	Endotoxin	0.001	Endotoxin	0.001	24	1	0	0/1
		0.01	„	0.01	24	1	0	0/1
		0.1	„	0.1	24	3	0	3/3
		1.0	„	1.0	24	3	0	3/3
		1.0	None	—		1	0	0/1
		2.5	„	—		2	0	0/2
		10.0	„	—		1	0	0/1
2	Jectofer	20	Endotoxin	0.01	6	1	0	0/1
		20	„	0.1	6	1	1	0/1
		20	„	1.0	6	1	0	0/1
		20	„	2.5	6	1	0	0/1
3	Jectofer	40	Endotoxin	0.1	3	1	1	0/1
		40	„	1.0	3	2	1	0/2
4	Jectofer	40	Endotoxin	0.1	6	1	1	0/1
		40	„	1.0	6	1	1	0/1
5	Iroject	20	Endotoxin	0.1	3	1	1	0/1
		20	„	1.0	3	1	1	0/1
6	Iroject	40	Endotoxin	0.1	6	1	0	1/1
		40	„	1.0	6	1	1	0/1
		40	„	2.5	6	1	1	0/1
7	Macofer	40	Endotoxin	0.1	6	1	1	0/1
		40	„	1.0	6	2	1	1/2
8	Imferon	40	Endotoxin	0.1	6	1	0	0/1
		40	„	1.0	6	2	2	2/2

Bilateral cortical necrosis of the kidneys was found when Iroject, Macofer, Imferon and A 2152 were used as preparing agents. No renal lesion was found in animals given Jectofer. The incidence of bilateral cortical necrosis is given in Tables 1, 2 and 3. Grossly the kidneys were enlarged and showed a heterogenous colour with alternate necrotic and haemorrhagic areas (Fig. 1). Microscopically the kidneys displayed focal necrosis which was most pronounced in the cortex (Fig. 2). Necrobiotic

Table 2. *Experiment B:* Incidence of bilateral cortical necrosis in rabbits after intravenous injection of endotoxin or saline following intravenous administration of endotoxin or some parenteral iron preparations. Each group consisted of five male and five female rabbits.

Group	Treatment				Time between injections in hours	Mortality		Incidence of bilateral cortical necrosis	
	preparing agent	dose (mg/kg)	provoking agent	dose (mg/kg)		♂	♀	♂	♀
1	Endotoxin	1	Saline	—	24	0	0	0/5	0/5
2	Endotoxin	1	Endotoxin	1	24	0	1	1/5	3/5
3	Imferon	40	Saline	—	6	0	0	0/5	0/5
4	Imferon	40	Endotoxin	1	6	0	0	0/5	0/5
5	Macofer	40	Saline	—	6	1	0	0/5	0/5
6	Macofer	40	Endotoxin	1	6	3	3	2/5	1/5
7	Iroject	40	Saline	—	6	0	0	0/5	0/5
8	Iroject	40	Endotoxin	1	6	2	1	3/5	0/5
9	Jectofer	30	Saline	—	6	0	0	0/5	0/5
10	Jectofer	30	Endotoxin	1	3	1	0	0/5	0/5
11	Jectofer	30	Endotoxin	1	6	2	0	0/5	0/5

Table 3. *Experiment C:* Incidence of bilateral cortical necrosis in rabbits after intravenous injection of endotoxin or saline following intravenous administration of endotoxin and A 2152. Each group consisted of five male and five female rabbits.

Group	Treatment				Time between injections in hours	Mortality		Incidence of bilateral cortical necrosis	
	preparing agent	dose (mg/kg)	provoking agent	dose (mg/kg)		♂	♀	♂	♀
1	Endotoxin	1	Endotoxin	1	24	1	2	4/5	3/5
2	A 2152	40	Saline	—	6	0	0	0/5	0/5
3	A 2152	40	Endotoxin	1	6	1	2	3/5	0/5

Table 4. Incidence of hepatic changes in rabbits after intravenous injection of endotoxin or saline following intravenous administration of endotoxin or A 2152. Each group consisted of five male and five female rabbits (Experiment C).

Group	Preparing agent	Provoking agent	Incidence of focal necrosis		Incidence of thrombosis	
			♂	♀	♂	♀
1	Endotoxin	Endotoxin	5/5	3/5	5/5	4/5
2	A 2152	Saline	0/5	2/5	0/5	0/5
3	A 2152	Endotoxin	2/5	3/5	0/5	0/5

nuclear changes and some fine fat droplets were observed in the glomeruli and tubular epithelium. There were often numerous haemorrhages in the renal tissue. Fibrin deposits were demonstrated in the blood vessels, mainly in the glomerular capillaries (Fig. 2). No iron-containing precipitates were found in blood vessels.

In experiment C examination of the liver was included. Slight focal necrosis was observed in all groups being most frequent when endotoxin was used as preparing agent (Table 4). Grossly the focal necrosis appeared as scattered small yellowish well-defined areas. Histologically the necroses were well-defined, of various sizes and with a round or, if they were larger, of a more irregular shape. The necroses mainly showed a mid-zonal distribution and were partly infiltrated with inflammatory cells mostly granulocytes. A fine droplet fatty change of the hepatocytes was found adjacent to the necrotic areas. Mixed microthrombi were demonstrated in the blood vessels after using endotoxin as preparing agent (Fig. 3). No microthrombi were, however, observed when A 2152 was given as preparing agent.

DISCUSSION

Bilateral cortical necrosis of the kidneys is considered as the identifying lesion of the generalized Shwartzman reaction (*Thomas & Good* 1952 b, *Krecke* 1964). Such a lesion was demonstrated in this study. The finding thus indicates that iron preparations can replace bacterial endotoxin as preparing agent in the rabbit. The results confirm earlier observations concerning saccharated oxide of iron in this respect (*Smith et al.* 1953, *Zweifach et al.* 1957). The generalized Shwartzman reaction was

produced by involvement of Imferon, Macofer, Iroject and A 2152 but not of Jectofer. A generalized Shwartzman-like reaction, however, has been found in pregnant rats given Jectofer (*Eriksson et al.* 1974). It should be noticed that Jectofer is a low-molecular compound in contrast to the other preparations. It might therefore be possible that high-molecular iron compounds are more prone to prepare the animals for elicitation of the generalized Shwartzman reaction than low-molecular ones.

When comparing the incidence of the generalized Shwartzman reaction in the three experiments, it seems as if it was easier to induce the reaction in experiment A in which rabbits of conventional type were used than in experiments B and C which were performed on SPF animals. The reason for this is unclear and emphasizes that caution must be exercised when comparing results from studies performed on different occasions.

When saccharated iron oxide is administered intravenously to rabbits, iron-containing precipitates can be formed in the blood, occlude glomerular capillaries, and give rise to acute renal lesions (*Ellis* 1956). In the present study no iron was observed in the deposits in the blood vessels, and no bilateral cortical necrosis was found in the kidneys of the control animals. The observations thus indicate that the renal lesions are not related to iron-containing precipitates in the blood.

When producing the generalized Shwartzman reaction by endotoxin, hepatic changes as focal and midzonal necrosis and thrombosis are well known characteristics. Such changes were also found in this study. However, differences between endotoxin and A 2152 were apparent in this respect. When A 2152 was used as preparing agent, the liver showed focal necrosis but no thrombosis. The observation might suggest that various mechanisms are involved in the induction of the generalized Shwartzman reaction, if an endotoxin or an iron compound is used as a preparing agent. Mechanisms involving vasomotor, thrombotic and metabolic factors are considered to be implicated in the development of the generalized Shwartzman reaction produced by endotoxin (*McKay* 1963, *McKay & Wong* 1963). It may be questioned whether hypotension plays a role in the induction of the observed hepatic changes. Focal and midzonal necrosis have been found in cases with continuous low blood pressure and poor general circulation (*Himsworth* 1948, *Magnusson* 1963). In this respect further studies are, however, needed.

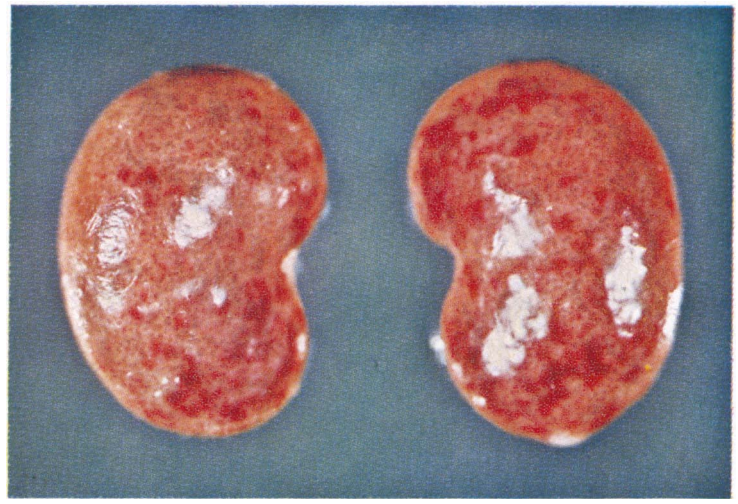

Figure 1. Cortical necrosis and haemorrhage in the kidney of a rabbit after intravenous injection of endotoxin following intravenous administration of Iroject.

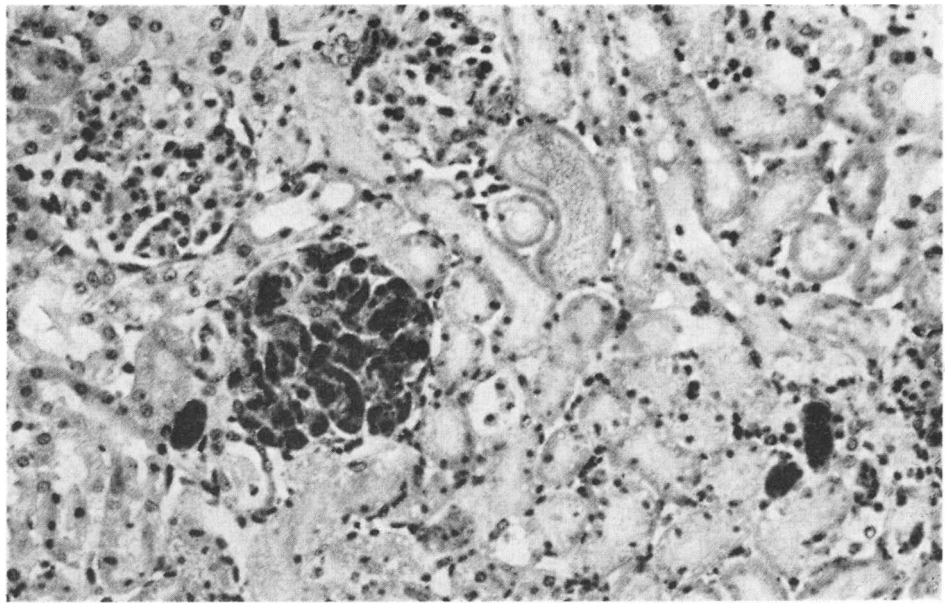

Figure 2. Cortical necrosis in the kidney of a rabbit after intravenous injection of endotoxin following intravenous administration of Iroject. Fibrinous deposits in the capillaries, mainly in one glomerulus. Lendrum's acid picro-Mallory method. × 300.

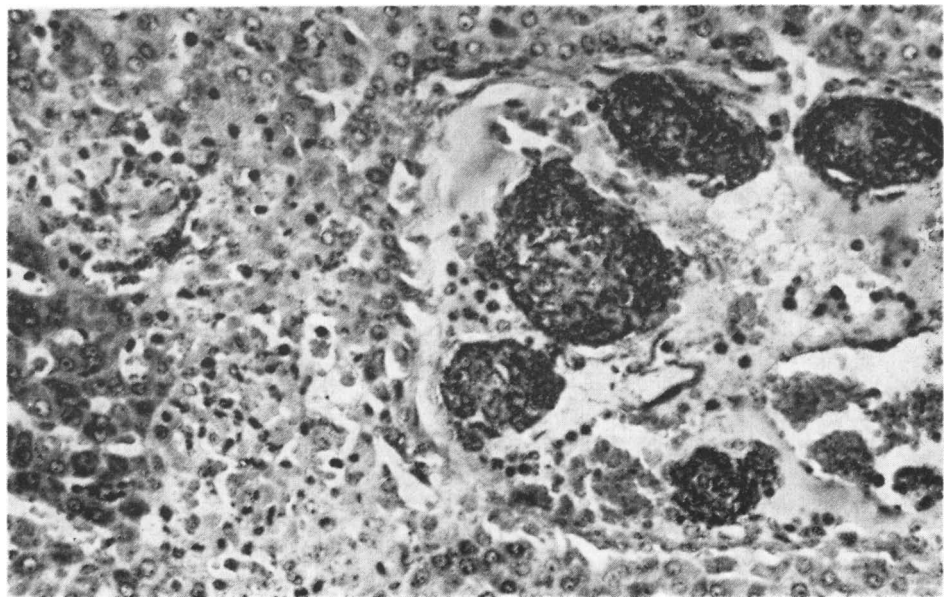

Figure 3. Necrosis (to the left) and thrombosis (to the right) in the liver of a rabbit after intravenous injection of endotoxin following intravenous administration of endotoxin. Lendrum's acid picro-Mallory method. × 300.

It is evident that iron compounds can act as preparing agents in the rabbit. The significance of iron complexes for the development of the generalized Shwartzman reaction in man and animals is at present unknown. There is no information on iron involvement in those reported cases of bilateral cortical renal necrosis which have been interpreted as a generalized Shwartzman reaction (*Krecke, Nordstoga* 1967, *Marcussen & Asnaes* 1972).

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SAMMANFATTNING

Utlösning av den generella Shwartzmanreaktionen med parenterala järnpreparat och bakteriellt endotoxin hos kaniner.

Några olika järnpreparat undersöktes beträffande deras förmåga att vara "preparing agents" vid utlösandet av den generella Shwartzmanreaktionen hos kanin. Som uttryck för den generella Shwartzmanreaktionen påvisades bilateral kortikal nekros i njurarna när Imferon, Iroject, Macofer och A 2152 användes som "preparing agents". Ingen njurskada iakttogs, när Jectofer användes.

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