J. Pharm. Pharmacol., 1965, **17**, 10–14 Received July 27, 1964

Effect of adrenalectomy on cotton pellet granuloma formation in the rat

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The development of granulomas over 3 days was inhibited in adrenalectomised rats when cotton pellets were implanted on the day of adrenalectomy and was potentiated when the pellets were implanted 7 days after adrenalectomy. The initial inhibitory effect of adrenalectomy was not observed when the granulomas were allowed to remain *in situ* for 7 days instead of 3 days and was completely reversed by treatment with corticosterone.

ALTHOUGH the actions of adrenal hormones on developing granulation tissue are widely known, there is some confusion about the effect of adrenalectomy upon healing wounds and granuloma formation. Whereas it has been reported that healing was retarded in adrenalectomised animals (Selye, 1947; Clayton & Prunty, 1951) and that the formation of granulomas around turpentine-induced abscesses in the rat was inhibited by adrenalectomy (Taubenhaus & Amromin, 1950), other workers have found that the amount of tissue formed around an implanted ivalon sponge (Pernokas, Edwards & Dunphy, 1957) or around a plastic ring (Jorgensen, 1962) in the rat was unaffected by adrenalectomy. Further reports record an increase in the amount of collagen produced in carrageenin-induced granulomas in adrenalectomised guinea-pigs (Robertson & Sanborn 1958) and an increase in the weight of carrageenin granulomas from adrenalectomised rats (Atkinson, Jenkins, Tomich & Woollett, 1962) compared with intact controls.

Our observations show that effects of adrenalectomy ranging from inhibition to potentiation of granuloma formation can be elicited in the rat and are dependant upon the time that elapses between adrenalectomy and implantation of the irritant material.

Experimental

METHODS

Female Wistar rats, 130 and 180 g weight, were bilaterally adrenalectomised through a dorsal incision under ether anaesthesia. Controls were sham operated and non-operated animals; the latter were given ether only. The room temperature was 27° and the adrenalectomised animals received 0·9% saline to drink instead of tap water. Sterile cotton pellets each weighing between 6 and 10 mg (Johnson and Johnson) were implanted one in each groin under ether anaesthesia. Each batch of pellets was screened for irritancy since they varied in this property. The time of implantation in relation to adrenalectomy varied and is stated in the text (day 0 = implantation immmediately after adrenalectomy).

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The granulomas were removed 3 days after implantation of the pellet since they reach maximum weight at about this time (Penn & Ashford, 1963). In one experiment, granulomas were removed after 7 days. The granulomas from each rat were immediately weighed wet in pairs and in some instances after being dried overnight at 60°. The dry weights quoted are those of the dry tissue only.

DRUGS

Hydrocortisone acetate was administered as a saline suspension by a single i.p. injection immediately after implantation of the pellets. Controls received saline.

Corticosterone was dissolved in 5% ethanol. 8 i.p. injections each of 100 μg/kg, were given during the 3 day test period as follows: day 0, 1 injection immediately after implantation; day 1, 3 injections; day 2, 3 injections; day 3, 1 injection 2 hr before death. Controls received 5% ethanol (0·1 ml/100g i.p.).

Results

3 DAY OLD GRANULOMAS FROM COTTON PELLETS IMPLANTED AT 0, 3, 5 AND 7 DAYS AFTER ADRENALECTOMY

The weight of granulomas from adrenalectomised rats was dependant upon the time that elapsed between adrenalectomy and implantation of

TABLE 1. WEIGHT OF 3 DAY OLD GRANULOMAS IMPLANTED AT 0, 3, 5 AND 7 DAYS AFTER ADRENALECTOMY

Group	No. of rats	Day of implantation	Mean paired granuloma wt. mg ± s.e.			
			Wet	P	Dry	P
Non-operated	6	0	413·7 ± 18·1		—	
Sham-operated	10	0	367·4 ± 13·1	<0·001	—	
Adrenalectomised	10	0	290·4 ± 13·2		—	
Sham-operated ..	10	0	363·1 ± 19·8	NS	57·9 ± 4·9	<0·01 *
Adrenalectomised	10	0	306·9 ± 23·2		34·9 ± 4·5	
Sham-operated	12	0	448·7 ± 16·4	<0·001	—	
Adrenalectomised	12	0	267·8 ± 8·0		—	
Non-operated	6	0	519·2 ± 42·6	<0·001	—	
Adrenalectomised	6	0	281·4 ± 17·4		—	
Non-operated	6	0	345·6 ± 19·1	<0·01	—	
Adrenalectomised	6	0	217·3 ± 24·2		—	
Non-operated	12	3	296·6 ± 17·7		42·8 ± 5·4	
Sham-operated	12	3	267·1 ± 19·1	NS	32·9 ± 13·9	NS
Adrenalectomised	12	3	306·6 ± 29·1		41·9 ± 6·8	
Sham-operated ..	12	5	281·8 ± 19·4	NS	40·5 ± 6·0	
Adrenalectomised	11	5	335·7 ± 26·2		49·2 ± 5·7	
Non-operated	24	7	341·4 ± 13·7		50·0 ± 3·9	
Sham-operated	24	7	375·7 ± 14·9	<0·001	57·4 ± 4·3	NS <0·01
Adrenalectomised	24	7	481·1 ± 24·6		68·8 ± 5·3	
Non-operated	10	7	477·5 ± 22·4		71·7 ± 5·3	*
Sham-operated	10	7	493·1 ± 34·8	<0·05	83·9 ± 9·5	NS <0·01
Adrenalectomised	10	7	600·9 ± 27·1		109·4 ± 8·4	

* Abstracted from Table 2, all rats having received control injections of 5% ethanol.
NS. Not significant P>0·05.

the pellets. Whereas the wet and dry weight was reduced by adrenalectomy when the pellets were implanted on day 0, there was statistically no significant difference in weight between adrenalectomised and intact groups when pellets were implanted on days 3 and 5. When the period between adrenalectomy and implantation was increased to 7 days the wet weight of granulomas from adrenalectomised rats was significantly greater than that of granulomas from sham-operated controls. However, the dry weight of the adrenalectomised group was not increased significantly ($P > 0.05$) compared with sham-operated controls but was significantly greater than non-operated controls ($P < 0.01$).

EFFECT OF CORTICOSTERONE ON 3 DAY OLD GRANULOMAS (Table 2)

Corticosterone treatment significantly increased the weight of granulomas from adrenalectomised rats implanted on day 0, the increase being

TABLE 2. EFFECT OF PARENTERAL INJECTIONS OF CORTICOSTERONE (8 × 100 μG/KG I.P.) ON THE WEIGHT OF 3 DAY OLD GRANULOMAS FROM COTTON PELLETS IMPLANTED AT 0 AND 7 DAYS AFTER ADRENALECTOMY

Group	No. rats	Day of implantation	Mean paired granuloma wt. mg ± s.e.			
			Wet	P	Dry	P
Sham-operated	10	0	363·1 ± 19·8	NS	57·9 ± 4·9	<0·05
Sham-operated + corticosterone	10	0	313·6 ± 23·4		39·3 ± 5·9	
Adrenalectomised	10	0	306·9 ± 23·2	<0·02	34·9 ± 4·5	<0·01
Adrenalectomised + corticosterone	10	0	422·8 ± 33·2		61·6 ± 7·3	
Non-operated	10	7	477·5 ± 22·4	<0·001	71·7 ± 5·3	<0·001
Non-operated + corticosterone	10	7	255·9 ± 8·6		42·3 ± 3·4	
Sham-operated	10	7	493·1 ± 34·8	<0·001	83·9 ± 9·5	<0·001
Sham-operated + corticosterone	10	7	249·3 ± 11·7		33·2 ± 3·1	
Adrenalectomised	10	7	600·9 ± 27·1	NS	109·4 ± 8·4	NS
Adrenalectomised + corticosterone	10	7	517·9 ± 32·2		94·2 ± 8·9	

38% in the wet and 76% in the dry granuloma weight. The same dose of corticosterone had an anti-inflammatory action in sham-operated rats and reduced the dry granuloma weight by 32% ($P < 0.05$); the wet granuloma weight was also reduced (13%), although this reduction was not significant ($P > 0.05$).

When cotton pellets were implanted on day 7, corticosterone slightly reduced the weight of granulomas in the adrenalectomised group although the reduction was not significant ($P > 0.05$). There was a marked anti-inflammatory action in intact rats, the reductions being non-operated 46% wet and 41% dry, sham-operated 49% wet and 60% dry.

SEVEN DAY OLD GRANULOMAS FROM COTTON PELLETS IMPLANTED ON DAY 0

There was no difference between wet granuloma weights from sham-operated controls and adrenalectomised rats when the pellets remained *in situ* for a period of 7 days. For groups of 10 animals the figures were

337·9 ± 20·8 and 361·2 ± 26·6 for adrenalectomised and sham operated rats respectively.

ANTI-INFLAMMATORY ACTION OF HYDROCORTISONE ACETATE ON GRANULOMAS FROM PELLETS IMPLANTED ON DAY 0 (Fig. 1)

Relatively high doses of hydrocortisone exerted an anti-inflammatory action in adrenalectomised and in intact animals. The dose-response curves were parallel over the dose-range 25·0–50·0 mg/kg.

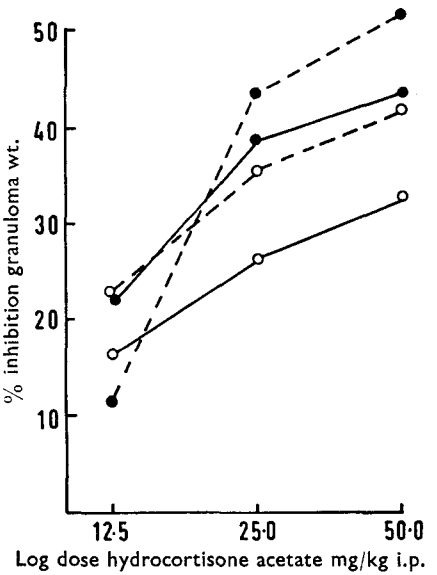

FIG. 1. The effect of hydrocortisone acetate on granulomas from cotton pellets implanted at the time of adrenalectomy and removed 3 days later. There were 8 rats per group. ●——● Non-operated wet weight. O——O Adrenalectomised wet weight. ● - - - ● Non-operated dry weight. O - - - O Adrenalectomised dry weight.

Discussion

Two clearly different effects of adrenalectomy have been shown—an inhibition of granuloma formation in acutely adrenalectomised rats and an increase in granuloma formation in rats implanted with cotton pellets 7 days after adrenalectomy. The transition stage was seen when implantation was on the 3rd and 5th day after adrenalectomy, the adrenalectomised rats responding in a similar manner at these times to intact controls. It is possible that much of the confusion in the literature about the effect of adrenalectomy on the formation of granulation tissue stems from this biphasic response to adrenalectomy.

The initial reduction in granuloma weight caused by adrenalectomy was reversed by small quantities of corticosterone which, however, had an anti-inflammatory action in intact rats. Presumably the different effect of corticosterone in adrenalectomised and intact rats was due to the small

dose used, all of it being needed in the former group of animals to correct the metabolic defect resulting from adrenalectomy and none being surplus for anti-inflammatory purposes. When a large excess of corticosteroid, in this case, hydrocortisone, was given to adrenalectomised rats an anti-inflammatory action was observed.

The inhibitory effect of adrenalectomy on pellets implanted on day 0 was not seen if these pellets were left *in situ* for 7 instead of 3 days.

A rebound increase in inflammatory response became evident after a delay of between 5 and 7 days following adrenalectomy. The delay suggests that some factor other than a simple deficiency of corticosteroids is involved. Long & Miles (1950) described a similar rebound effect in the response of intact immunised guinea-pigs to tuberculin after withdrawal of cortisone treatment. The increase in hypersensitivity in this instance was also preceded by a delay of several days and it was suggested that an increased secretion of thyroxine was responsible, this hormone having an action opposed to that of cortisone. Since thyroxine has been shown to promote the development of granulation tissue in hypophysectomised rats (Taubenhaus & Amromin, 1950) although not in intact animals (Taubenhaus, Taylor & Morton, 1952) it is possible that it may be implicated in the increased inflammatory response 7 days after adrenalectomy. On the other hand Spencer & West (1963), who studied the relationship between the thyroid and adrenal glands and their effect on histamine metabolism in the rat, concluded that there was no true balance between the glands, the anti-cortisone action of thyroxine being indirect and mediated via an alteration in the amount of corticosteroid available to the tissues.

It is likely that the stress of surgery in sham-operated control animals would result in an initial hypersecretion of corticosteroids which may affect the development of granulomas after implantation on day 0. In fact the weight of granulomas from sham controls after implantation on days 0 and 3 was less than those of non-operated controls although P exceeded 0·05 in both instances. Similarly, after implantation on day 7, sham-operated animals occupied an intermediate position between non-operated and adrenalectomised rats in respect of granuloma weight and showed a greater response to the anti-inflammatory effects of corticosterone than non-operated controls.

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