

THE EFFECT OF IRRITANT SUBSTANCES ON THE DEPOSITION OF GRANULATION TISSUE IN THE COTTON PELLET TEST

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The observation that in a group of substances derived from liquorice, anti-inflammatory activity as determined by the cotton pellet test seemed to be associated with irritant effects at the site of injection, led to the investigation of the effect of known irritant substances. It was found that tartar emetic, croton oil, talc and animal charcoal caused a significant reduction in the granulation tissue deposited around cotton pellets implanted in both intact and adrenalectomised rats. It is suggested that there is some limit to the total number of leucocytes which, in the course of a few days, can be mobilised to take part in an inflammatory reaction. Thus the two sites at which irritant effects have been produced, i.e. the site of implantation of the cotton pellets and the site of injection of the irritant substance, compete for these cells, giving apparently an "anti-inflammatory" effect. This may well represent an important disadvantage of the cotton pellet test, making it suitable only for the investigation of non-irritant substances.

In the course of investigating the anti-inflammatory activity of a series of substances derived from liquorice by the cotton pellet test, it was noted that the substances which had a marked anti-inflammatory effect also caused a considerable amount of irritation at the site of injection, whereas compounds which were less potent did not have any adverse local effects (Cygielman, 1963). Most of the compounds in this series were insoluble and, in addition to the increased vascularization apparent at the site of injection, there was a massive influx of white cells. It was of interest to determine the extent to which the anti-inflammatory activity of the compounds tested was associated with their irritant effects. Since there is a possibility that a stress reaction may be produced by the administration of these substances, and that this may be responsible, to some extent at least, for the increased anti-inflammatory effect, experiments were made in adrenalectomised as well as in intact animals.

METHODS

A modification of the method of Meier, Schuler and Desaulles (1950) for the measurement of anti-inflammatory activity was used. Experiments were done on male Wistar rats, weighing 130–190g. Each experiment included one or more treated groups and a control group, and there were five rats in each group. The cotton pellets were weighed individually, and only those within 0.2 mg. of the mean weight were used for any one experiment. The pellets were sterilised by heating for 2 hr. at 150° and implanted subcutaneously, one in each axilla and groin. On the fourth day after implantation the cotton pellets were dissected out, dried for 24 hr. at 60°, and re-weighed. The increase in weight was used as an indication of

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the amount of granulation tissue deposited. Test substances were injected subcutaneously daily for four days unless otherwise stated, beginning on the day of implantation. The substances were given as solution or suspension in 0.1 to 1.0 ml. of water, except croton oil, which was injected as such or dissolved in olive oil. Controls received 1 ml. of saline.

The anti-inflammatory effect was calculated as the percentage reduction of the increase in weight of the cotton pellets in the treated as compared with the control group.

The substances chosen for administration were tartar emetic, digitoxin, croton oil, talc and animal charcoal, i.e. substances known to have irritant properties when administered subcutaneously, but believed not to have any anti-inflammatory activity.

Adrenalectomy was performed under ether anaesthesia through a median dorsal skin incision; a slit was made in the body wall over the anterior pole of each kidney, and the adrenal glands were carefully removed by means of curved forceps. Suture clips were used to close the wound. Implantation of pellets was performed at least six days later, and the completeness of the adrenalectomy was confirmed at post-mortem examination.

RESULTS

Local Effects at the Site of Injection

Tartar emetic. 0.1 ml. of a 5 per cent solution/rat/day was found to produce a considerable local inflammatory reaction, consisting mainly of increased vascularisation, and the formation of a yellow watery exudate. This dose was toxic, since in one experiment one out of five intact (i.e. non-adrenalectomised) animals died after the third injection, and in another experiment all adrenalectomised rats died within 24 hr. of a single injection.

At a dose level of 0.03 ml. of the 5 per cent, or 0.1–0.2 ml. of a 1 per cent solution, the local inflammatory effects were greatly reduced, with only an increase in the number of capillaries to mark the site of injection.

Digitoxin. This was administered at two dose levels, 0.5 ml. and 1.0 ml. of a 0.1 per cent aqueous suspension/rat/day. The irritant effect was very small, consisting of some increased vascularization; there was no difference in the effect produced by the two dose levels.

Croton oil. When a single dose of 0.1 ml. of croton oil was injected, the whole surrounding area became grossly necrosed, with endurance of the tissues and sloughing; there were also systemic toxic effects, with the rats losing weight as compared with the control group. When 0.2 ml. of a 10 per cent solution in olive oil was given, again as a single dose, marked necrosis was found at the site of injection, but the toxic effects were reduced, the rats gaining weight, though one out of five in the adrenalectomised group died.

Talc. When administered at a dose level of 10 mg./rat/day, talc greatly increased local vascularity and induced the formation of granulation tissue, the talc finally becoming completely enclosed by the latter. All these effects were increased when the dose was raised to 50 mg./rat/day.

Animal charcoal. The effects were similar to those produced by talc, though the vascularization was more pronounced. 25 and 50 mg./rat/day caused approximately the same degree of irritation.

Effect on the Deposition of Granulation Tissue around the Cotton Pellets.

The results obtained are given in Table I and summarised in Table II. As can be seen, digitoxin, which had only a slight inflammatory effect, did not affect the weight of granulation deposited. All the other substances, at dose levels producing a marked effect at the site of injection, also had a marked anti-inflammatory activity not greatly modified by adrenalectomy.

TABLE I

THE EFFECT OF SUBCUTANEOUS ADMINISTRATION OF IRRITANT SUBSTANCES ON THE AMOUNT OF GRANULATION TISSUE DEPOSITED IN THE COTTON PELLET TEST

(Results are expressed as the percentage reduction in the gain in weight of the cotton pellets. All doses are given as the amount of substance injected/rat/day, with the exception of croton oil, which was given as a single dose. There are five animals in each group. The significance is that between the treated group and the control group which was present in each experiment.)

Test No.	Treatment		Intact or adrenalectomised rats	Reduction of granulation tissue per cent	P
	Compound	Dose			
1*	Tartar emetic	0.1 ml. 5 per cent	Intact	31	0.005
2	"	0.03 ml. 5 per cent	"	20	0.01
3	Digitoxin	0.5 ml. 0.1 per cent	"	10	0.02
	"	1.0 ml. 0.1 per cent	"	0	> 0.1
4	Croton Oil	0.1 ml.	"	49	0.001
5	"	0.1 ml.	"	28	0.02
	"	0.1 ml.	Ad.	27	0.01
	Tartar emetic	0.1 ml. 5 per cent	Intact	28	0.01
6	"	0.1 ml. 1 per cent	"	9	—
	"	0.1 ml. 1 per cent	Ad.	0	—
	Talc	10 mg.	Intact	8	—
	"	10 mg.	Ad.	0	—
	Talc	50 mg.	Intact	44	< 0.001
	"	50 mg.	Ad.	49	< 0.002
	Croton Oil	0.2 ml. 10 per cent	Intact	18	0.02
	"	0.2 ml. 10 per cent	Ad.	21	0.05
	Tartar emetic	0.2 ml. 1 per cent	Intact	10	0.01
	"	0.2 ml. 1 per cent	Ad.	27	0.001
8	Animal charcoal	25 mg.	Intact	30	0.001
9	"	50 mg.	"	26	0.001
	"	50 mg.	Ad.	17	0.02

* One animal died after the third injection

DISCUSSION

Since tartar emetic is absorbed from the site of injection, and has at one time been used as an anti-inflammatory agent, and since the composition of croton oil is not certain, and it may have a component which is absorbed, the results obtained with these substances might not be considered convincing. However, neither charcoal nor talc are absorbed, nor are they known to possess any pharmacological activity when given systemically, and it is thus their effects which are of the greatest interest.

It was thought that the most probable explanation of these effects would be that the injection of irritant substances elicited a stress reaction. It has been previously demonstrated by Kellett (1959) that cold stress decreases the amount of granulation tissue formed, and he attributed this to the release of glucocorticoids from the adrenal cortex. However, the

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fact that substances which are apparently devoid of any systemic effect nevertheless have an anti-inflammatory action in bilaterally adrenalectomised animals indicates that a different mechanism must be responsible for the effects so obtained.

Both talc and animal charcoal caused extensive infiltration with granulation tissue at the site of injection. In the case of talc this effect was more marked with the larger dose.

The explanation of the apparent anti-inflammatory effect of these substances offered here is that there may be some limit to the amount of granulation tissue which an animal can produce in four days, and when a strong irritant stimulus is produced at the site of injection, the granulation tissue is deposited there rather than at the site of implantation of the cotton pellets. It could be said that the cotton pellets and the irritant substances are competing for the total number of macrophages available for the inflammatory reaction.

The relative intensity of the inflammatory reaction at the site of injection determines the amount of granulation tissue deposited around the cotton

TABLE II
SUMMARY OF THE RESULTS IN INTACT AND ADRENALECTOMISED RATS

Compound	Dose	Reduction of granulation tissue per cent	
		Intact rats	Adrenalectomised rats
Tartar emetic	0.1 ml. 5 per cent	31	28
	0.03 ml. 5 per cent	20	—
	0.1 ml. 1 per cent	9	0
	0.2 ml. 1 per cent	10	27
Croton oil	0.1 ml.	49	28
	0.2 ml. 10 per cent	18	21
Animal charcoal	25 mg.	30	—
	50 mg.	26	17
Talc	10 mg.	8	0
	50 mg.	44	49

• All animals died.

pellets: thus 50 mg./rat/day of talc had marked irritant and anti-inflammatory effects both in intact and adrenalectomised rats, while 10 mg./rat/day, which had less irritant effect, showed only a weak anti-inflammatory effect in intact rats, and none in adrenalectomised rats. It is suggested that the effect obtained in these experiments are rather similar to those obtained when the body is overwhelmed by bacteria and cannot cope with them.

This may well represent a serious criticism of the cotton pellet test as a means of estimating anti-inflammatory activity, since it means that it is only suitable for substances which are relatively non-irritant, whereas the effects obtained with irritant substances may be due to an artefact.

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