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PRODUCTION OF AN ANTI-INFLAMMATORY SUBSTANCE AT A SITE OF INFLAMMATION

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The anti-inflammatory effect of various substances was measured in rats implanted with cotton wool pellets. Injection or implantation of irritant materials decreased the deposition of granulation tissue on the pellets. As the amount of irritant material increased there was a corresponding decrease in the amount of tissue deposited on the pellets. There is evidence that this could not be explained by a limit to the amount of granulation tissue available within the body, and competition for it between the cotton pellets and the implanted substances. An alternative hypothesis that an anti-inflammatory substance is produced at the site of irritation (the site of implantation of the irritant substance, such as polyester sponges) was investigated. The evidence obtained supports this hypothesis. Inflammatory exudate, squeezed from polyester sponges which had been implanted subcutaneously in the backs of adrenalectomized rats. The substance responsible for this effect is probably not a steroid, and is not normally present in the plasma of adrenalectomized animals. There is, however, some (although not conclusive) evidence that it is present in the plasma of animals in which sponges have been implanted. It does not appear to be produced by incubation *in vitro* of plasma with sponge. The significance of these observations is discussed.

It has previously been shown (Cygielman & Robson, 1963) that an anti-inflammatory effect can be demonstrated in the cotton pellet test by the use of substances which produce an irritant action at the site of injection. Such substances include talc, charcoal and tartar emetic, and the reduction in the amount of granulation tissue deposited on the pellets occurs in both intact and adrenalectomized animals. The explanation suggested was that irritant substances compete for the limited number of leucocytes which the body can provide during the 4 days of the test, and that the anti-inflammatory effect thus produced is an artefact. An alternative explanation is that a substance is produced locally at the site of injection of the irritant material, and that the substance is absorbed into the circulation and can then act in other parts of the body to reduce the inflammatory reaction. Laden, Blackwell & Fosdick (1958) have presented evidence for the production of such a humorally transmitted substance, and have shown that pleural inflammation induced by the intrapleural injection of an irritant substance can be reduced by creating an additional site of inflammation in the leg. It has, moreover, been shown that many pharmacologically active substances are released locally following injection of chemical irritants (Spector & Willoughby, 1957, 1959, 1962). However only the short-term actions of such substances on capillary permeability have been considered and not their more prolonged effects on the cellular reactions.

The present investigation provides evidence which suggests that the anti-inflammatory effect produced by injecting irritant substances cannot be explained on the hypothesis that there is a limit to the inflammatory reaction of the body. It is in favour of the view, however, that a chemical substance (or substances) is released at the site of irritation produced by implantation of polyester sponges, and that the substance has anti-inflammatory properties in other parts of the body.

METHODS

Measurement of anti-inflammatory effects

Anti-inflammatory activity was measured by a modification of the method of Meier, Schuler & Desaulles (1950). All the experiments were performed on male Wistar rats weighing 140 to 200 g. The rats were divided into groups so that the mean weight of each group for any one experiment was the same. In general there were five animals in each group, although in some of the later experiments this number was increased. Cotton pellets were weighed individually on a torsion balance and only those within 0.25 mg of the mean were used for any one experiment. The pellets were sterilized by heating to 150° C for 2 hr and then implanted subcutaneously one in each axilla and groin, giving a total of four pellets per animal. On the 4th day after implantation they were dissected out, dried at 60° C for at least 24 hr, and reweighed. The increase in weight was used as an index of the amount of granulation tissue deposited. The anti-inflammatory effect was then calculated as the percentage reduction of the increase in weight of the cotton pellets in the treated group as compared with the control group. Extreme values were eliminated by the method of Dixon & Massey (1957), and the statistical significance of the difference between groups was calculated in each case by the use of Students' *t*-test.

Adrenalectomy

Adrenalectomy was performed during 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. The slits in the body wall were sutured with cotton, and the skin flaps closed with 12 mm metal suture clips. When adrenalectomy was performed it was always done a few minutes before implantation of pellets and/or sponges.

Implantation of sponges

Polyester sponge sheet was cut to the required size by means of a guillotine. The individual sponges were then sterilized by heating to 150° C for 2 hr. When adrenalectomy was required this was done through a posterior incision. The peritoneum was then closed and the sponges implanted subcutaneously. Again the skin flaps were closed with metal suture clips. Cotton wool rolls and charcoal tablets were implanted in a similar manner.

Collection of sponge exudate

In those experiments in which the inflammatory exudate was collected for reinjection intraperitoneally into other animals, the reinjection was done within a few hours after collection (with the exception of the preliminary experiment when the fluid was stored for not more than 14 days at −4° C). The sponges together with any adhering tissue were dissected out roughly and the fluid squeezed out manually. The resulting material was centrifuged at about 2,000 revs/min for 30 to 40 min in order to remove debris, including blood and tissue cells. Between these operations the fluid was kept in the refrigerator.

Collection of plasma

In some experiments the anti-inflammatory action of plasma was studied. In such experiments the donor animals were anaesthetized with ether, a median incision was made in the ventral abdominal wall, and the alimentary canal displaced to one side to expose the posterior vena

cava. Blood was withdrawn from this vessel into a 10 ml. syringe fitted with a No. 1 needle and containing 0.5 ml. of 3% sodium citrate. On average 5 to 6 ml. of blood were obtained from each rat. The samples of blood from all the animals in the same group were pooled and, after centrifuging at about 2,000 revs/min for 30 to 40 min, the plasma was removed with a Pasteur pipette. As with the sponge exudate, the plasma was injected into the recipient animals within a few hours after collection. Between the various operations the fluid was again kept in the refrigerator.

In vitro *incubation of plasma with sponge*

In this experiment the donor animals had been adrenalectomized 3 to 4 days earlier to allow for the elimination of circulating adrenal steroids. Plasma was obtained from the animals by the method described above, but in order to avoid infection certain precautions were observed. After anaesthetizing the rat, its lower half was dipped in a solution of 1% cetrimonium bromide in 90% alcohol, and the skin was opened with sterile scissors and forceps. Fresh pairs of sterile scissors and forceps were used to open the abdomen and expose the vena cava. Blood was withdrawn from each animal using a sterile 10 ml. disposable syringe and a No. 1 needle. Sterile 3.8% sodium citrate solution (0.5 ml.) was used as an anticoagulant. Blood was collected in sterile centrifuge tubes and after centrifugation the plasma was transferred to sterile bottles, adopting the usual sterile technique. These bottles and their contents were incubated at 37° C for 3 or 4 days before injection into recipient rats. Six groups of recipient rats were used to test the effects of incubating saline alone, saline with sponge, plasma alone, plasma with sponge, plasma and antibiotic, and finally plasma, antibiotic and sponge. Two pieces of polyester sponge were used, each measuring 25 × 20 × 6 mm, and were sterilized with the bottle. Each group of recipient rats received four daily injections of 2 ml. of these fluids. The experimental design was such that the samples of fluid had, by the time they were required for injection, been incubated for 3 or 4 days. The antibiotics used were chlortetracycline and benzylpenicillin to provide final concentrations of 0.05 mg/ml. and 50 U/ml. of plasma respectively. After the period of incubation each sample of plasma was tested for contamination on blood agar plates.

Materials used for implantation

The cotton wool pellets were manufactured by Johnson & Johnson Ltd., and only those within the general range of 5 to 10 mg were used. In addition a number of other materials were implanted as irritants, namely cotton wool rolls, charcoal tablets and polyester sponges. The cotton rolls were 1 cm in diameter and 4 cm long. The charcoal tablets were prepared by the Department of Pharmacy at Guy's Hospital, and contained 33.3 mg of charcoal and 16.7 mg of lactose. The resultant tablets were 5.7 mm in diameter and 2.5 mm thick. The polyester sponge was obtained as 0.25 in. thick sheet from the Defiance Rubber Co. Ltd., Croydon, but no information concerning its chemical nature is available. Two different samples were used in the experiments with similar results. It was cut to the required size on a guillotine and in all the experiments in which sponge exudate was produced pieces measuring 50 × 20 × 6 mm were used, having an overall surface area of 2,840 sq mm.

RESULTS

Evidence that the inflammatory reaction is not limited

Several experiments are presented which have been performed in both intact and adrenalectomized rats. They all suggest that an experimental limit to the granulation reaction is not the explanation for the anti-inflammatory effect following implantation of irritant substances.

Attempt to reach a limit using polyester sponges. Polyester sponges of various sizes were implanted subcutaneously one in each axilla and groin. After 4 days the sponges were removed, dried and the weight of tissue on each calculated. The

TABLE 1

THE EFFECT OF VARYING THE SIZE OF IMPLANTED SPONGES ON THE GRANULATION REACTION ROUND EACH SPONGE, AND THE TOTAL REACTION PER RAT

Each rat was implanted with four sponges and the last column thus shows the total increase for the four sponges implanted in each rat. The increases in dry weight of sponge are means with standard errors. Each result represents the average of five rats. The average initial body weight of the rats was 143 g

Dimensions of sponges (mm)	Surface area of sponges (sq mm)	Initial weight of single sponge (mg)	Increase in dry weight of sponge (mg)	Total increase in weight of dry tissue (mg/rat)
10 × 5 × 3	190	3·0	5·5 ± 0·4	22
20 × 2·5 × 3	235	3·0	5·8 ± 0·3	23
10 × 5 × 6	280	6·9	8·8 ± 0·7	35
20 × 5 × 3	350	6·8	9·9 ± 0·6	40
10 × 10 × 6	440	14·1	26·2 ± 1·5	105
20 × 5 × 6	500	14·5	15·8 ± 1·4	63
20 × 10 × 3	580	14·4	29·4 ± 1·0	117
20 × 10 × 6	760	29·5	40·0 ± 3·1	160

results are summarized in Table 1 and illustrated in Fig. 1. This shows that although a total of up to 160 mg of tissue has been deposited on the sponges in each animal, there is no sign of tailing off in the intensity of the reaction. Unfortunately larger reactions could not be observed by this technique because of the physical impossibility of implanting larger sponges in the axilla and groin.

Implantation of substances in addition to cotton pellets. Table 2 gives the results of four separate experiments in which different materials have been implanted sub-

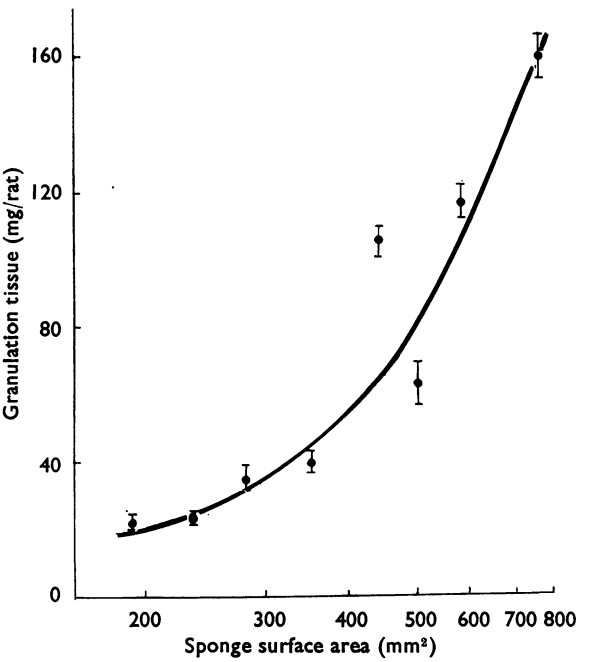

Fig. 1. Effect of varying the size of implanted sponges (abscissa, log scale) on the total granulation reaction per rat (ordinate). Each point represents the mean and standard error for five rats.

TABLE 2

EFFECT OF IMPLANTING IRRITANT MATERIALS SUBCUTANEOUSLY IN THE BACKS BOTH OF INTACT AND OF ADRENALECTOMIZED RATS ON THE GRANULATION REACTION AROUND COTTON WOOL PELLETS

Results, and the body weights, represent the averages for five rats. Increases in dry weight of pellets are means and standard errors. Intact = not adrenalectomized. Treatments refer to implantations per rat

Treatment	Initial pellet weight (mg)	Initial animal weight (g)	Increase in dry weight of pellet (mg)	Effect (%)	P	Increase in dry weight of irritant (mg/rat)
Sponge : intact						
Control	7·2	152	8·82 ± 0·67			
Sponge (50×10×6 mm)	7·2	152	5·69 ± 0·30	−36	<0·001	204
Sponge (50×20×6 mm)	7·2	152	5·40 ± 0·54	−39	<0·001	354
Sponge : adrenalectomized						
Control	6·7	166	7·45 ± 0·55			
Sponge (25×10×6 mm)	6·7	166	6·03 ± 0·41	−19	<0·05	96
Sponge (50×10×6) mm	6·7	166	6·23 ± 0·52	−16	<0·20	206
Sponge (50×20×6 mm)	6·7	166	5·39 ± 0·50	−32	<0·02	392
Charcoal tablets : intact						
Control	8·7	158	9·82 ± 0·94			
1 tablet	8·7	158	9·78 ± 1·05	0	—	73
2 tablets	8·7	158	8·19 ± 0·62	−17	<0·2	148
3 tablets	8·7	158	5·33 ± 0·34	−46	<0·001	244
Cotton wool rolls : adrenalectomized						
Control	8·2	173	10·91 ± 0·72			
½ roll	8·2	173	6·97 ± 0·65	−36	<0·001	131
1 roll	8·2	173	6·38 ± 0·56	−42	<0·001	219
2 rolls	8·2	173	4·87 ± 0·45	−55	<0·001	445
3 rolls	8·2	173	4·31 ± 0·45	−61	<0·001	745

cutaneously in the back, in addition to cotton pellets in the axilla and groin. On the 4th day in each instance both the material in the back and the cotton pellets were dissected out, dried and weighed. The results in both intact and adrenalectomized rats indicate that, as the size of the inflammatory reaction around the irritant in the back of animal increases (as indicated by the dry weight of tissue deposited on the material), so the inflammatory effect at the pellets decreases. This agrees with the results of Cygielman & Robson (1963). However, the amount of tissue deposited on the irritant substance in the back may increase by 100% or even 200% with only a 20 to 40% reduction in the amount of tissue deposited on the pellets. For example in the experiment in which cotton wool rolls were implanted an average of 131 mg of tissue was deposited on half a cotton roll. This produced a 36% reduction in the amount of tissue deposited on the pellets. However on two cotton wool rolls an average of 445 mg of tissue was deposited (a 240% increase) but tissue deposition on the pellets was only reduced by a further 19%. Similar effects could be seen in each of the three other experiments performed, using polyester sponge or charcoal tablets as the irritant material.

This result shows quite conclusively that a limit to the total inflammatory reaction within an animal does not explain the anti-inflammatory effects observed in cotton wool pellets following implantation of an irritant material elsewhere in the body.

Evidence for chemical transmission of the anti-inflammatory effect

The action of sponge exudate from adrenalectomized rats on the inflammatory reaction around cotton pellets. Fluid was obtained by squeezing sponges which had been implanted in adrenalectomized rats for 4 days. This fluid was injected daily for 4 days into recipient rats in which cotton pellets had been implanted, and caused a considerable reduction in the inflammatory reaction around the cotton pellets, as compared with saline-treated control animals. Table 3 presents the results of a

TABLE 3

THE EFFECT OF IMPLANTATION OF POLYESTER SPONGES ON THE COTTON PELLET REACTION IN ADRENALECTOMIZED RATS (DONORS), AND THE EFFECT OF DAILY INJECTIONS OF 1 ML. OF THE EXUDATE FROM THESE SPONGES ON THE INFLAMMATORY REACTION AROUND COTTON PELLETS BOTH IN INTACT AND IN ADRENALECTOMIZED RATS (RECIPIENTS)

*Twenty rats in this group, five rats in the other groups. I.p. = intraperitoneal ; s.c. = subcutaneous. Values are means for the groups. Increases in dry weight of pellets are means and standard errors

Treatment	Initial pellet weight (mg)	Initial animal weight (g)	Increase in dry weight of pellet (mg)	Effect (%)	*P*
Donors : adrenalectomized					
Control	8·2	176	8·99 ± 0·61		
Sponge (50 × 20 × 6 mm)*	8·2	176	6·36 ± 0·32	−29	<0·001
Recipients : intact					
Control	9·2	137	8·93 ± 0·62		
Sponge exudate (i.p.)	9·2	137	6·79 ± 0·57	−24	<0·02
Sponge exudate (s.c.)	9·2	137	6·45 ± 0·51	−28	<0·01
Recipients : adrenalectomized					
Control	5·2	190	6·54 ± 0·62		
Control exudate (s.c.)	5·2	190	4·79 ± 0·38	−27	<0·02

single experiment in which fluid was obtained from sponges, which had been implanted in twenty adrenalectomized donor animals. These animals had also received cotton pellets, and comparison of dry weights of tissue on these with that on pellets removed from control animals without sponges, confirmed the anti-inflammatory effect of the sponges (which in this experiment was 29%). About 4 ml. of fluid were squeezed from each sponge, giving a total of 80 ml. for the twenty donor animals. Of this fluid, 1 ml. was injected daily both into intact and into adrenalectomized rats in which cotton pellets had been implanted. When the fluid was given subcutaneously into intact animals this produced a 28% reduction ($P<0.01$) of the material deposited on the pellets; given intraperitoneally there was a 24% reduction ($P<0.02$); and given subcutaneously in adrenalectomized animals there was a 27% reduction ($P<0.02$). In each instance the effect was measured by comparison with saline-treated controls.

The action of plasma from adrenalectomized rats on the inflammatory reaction around cotton pellets. If an anti-inflammatory substance is produced at a site of inflammation (for example where a sponge is implanted) and this substance is then carried in the blood to another site (for example where cotton pellets are implanted)

to reduce the inflammatory response at the second site, then such a substance ought to be demonstrable in the plasma of such animals. Experiments were devised to determine whether this was so. Suitable controls were also arranged to show that plasma from adrenalectomized animals in which no sponges had been implanted was itself devoid of anti-inflammatory action.

Since the potential activity of the plasma is likely to be less than that of the fluid obtained from the sponge, the daily volume of fluid injected per animal was increased to 2 ml. Also, to avoid any possible deterioration in the activity of the fluid with time (a possibility indicated by two unsuccessful attempts to keep fluid), the samples both of fluid and of plasma were obtained from the donor animals on the day on which they were required for injection into the recipients.

The results of three entirely independent experiments are presented in Table 4. In all three experiments the sponge exudate, given daily in 2 ml. doses for 4 days, produced its characteristic anti-inflammatory effect, ranging in efficacy from 28 to 39% ($P<0.001$, as a combined value for the effect of 2 ml. of exudate in adrenalectomized animal). Plasma obtained from animals which had simply been adrenalectomized (without sponges implanted, and referred to in Table 4 as Plasma control)

TABLE 4

THE EFFECT OF FOUR DAILY INJECTIONS OF 2 ML. OF PLASMA OR OF SPONGE EXUDATE FROM ADRENALECTOMIZED DONOR RATS ON THE GRANULATION REACTION AROUND COTTON PELLETS IN BOTH INTACT AND ADRENALECTOMIZED RECIPIENT RATS

The percentage effects and significance values are obtained by comparison with saline-treated control rats. Plasma control refers to the plasma obtained from donor rats without implanted sponges, whilst Plasma test refers to plasma obtained from rats which had been implanted with sponges. Values are means for the groups of rats, and the increases in dry weights of pellets are means and standard errors. The anti-inflammatory effect of the sponges in the donor rats was measured in each instance by the simultaneous implantation of cotton pellets into these rats, and comparison of the dry weight of tissue on those pellets from rats with sponges with those from rats without sponges. Four groups of donor rats were used in each experiment (*a*, *b* and *c*). The anti-inflammatory effect of the sponges in each of these four donor groups showed some variation, but the mean anti-inflammatory effect was fairly constant, being respectively in the three experiments 29%, 27% and 34%

	Treatment	Initial pellet weight (mg)	Initial animal weight (g)	Increase in dry weight of pellet (mg)	Effect (%)	*P*
(*a*)	*Intact*					
	Saline control	7·7	212	9·91 ± 0·59		
	Plasma control	7·7	212	9·13 ± 0·79	−8	>0·5
	Plasma test	7·7	212	10·93 ± 0·91	+10	>0·4
	Sponge exudate (2 ml.)	7·7	212	7·13 ± 0·66	−28	<0·01
	Sponge exudate (1 ml.)	7·7	212	8·51 ± 0·79	−14	<0·2
(*b*)	*Adrenalectomized*					
	Saline control	8·7	191	11·48 ± 1·03		
	Plasma control	8·7	191	9·74 ± 0·46	−15	>0·1
	Plasma test	8·7	191	8·96 ± 0·44	−22	<0·05
	Sponge exudate (2 ml.)	8·7	191	6·96 ± 0·52	−39	<0·001
	Sponge exudate (1 ml.)	8·7	191	7·66 ± 0·60	−33	<0·01
(*c*)	*Adrenalectomized*					
	Saline control	8·2	188	9·35 ± 0·74		
	Plasma control	8·2	188	7·89 ± 0·50	−16	>0·1
	Plasma test	8·2	188	6·52 ± 0·49	−30	<0·002
	Sponge exudate (2 ml.)	8·2	188	5·96 ± 0·47	−36	<0·001

produced no significant reduction in the inflammatory reaction when compared with saline-treated controls ($P>0.05$, combined value for adrenalectomized recipients). However, plasma obtained from the same animals as the sponge exudate (referred to in Table 4 as Plasma test) produced a variable effect. When injected into intact animals (Table 4, *a*) no significant reduction was observed ($P>0.4$). When injected into adrenalectomized animals (Table 4, *b*) a 19% reduction was seen, compared with saline-treated controls, which was on the border-line of significance ($0.1>P>0.05$). On repeating this last experiment, however, using twice the number of animals (ten in each group instead of five) the reduction produced by the plasma was highly significant. Table 4, *c* shows that it produced a 30% reduction ($P<0.01$). The combined P value is also highly significant ($P<0.001$). These results are discussed later.

The action of plasma which had been incubated with sponge on the inflammatory reaction around cotton pellets. In order to exclude the possibilities that the anti-inflammatory substance had simply been extracted from the sponge, or that the sponge had catalysed a breakdown of the plasma leading to the production of an anti-inflammatory substance, sponge was incubated *in vitro* with plasma under sterile conditions for a period of 4 days. The resulting fluid was then tested for anti-inflammatory activity by injecting it into rats in which cotton pellets had been implanted. In order to eliminate circulating steroids, the plasma was obtained from animals which had been adrenalectomized 3 to 4 days earlier and, in order to avoid infection during the period of incubation, sterile precautions were observed during its collection. The results (Table 5) show firstly that an active anti-inflammatory substance is not extractable from the sponge following incubation with saline; and secondly that polyester sponge does not catalyse a breakdown of plasma to produce an anti-inflammatory substance, either in the presence or absence of antibiotics. Antibiotics were used as a precaution to minimize the likelihood of infection which might have produced an unusual result. However, in no instance did infection occur, as indicated by growth on blood-agar plates.

TABLE 5

THE EFFECT OF PLASMA, WHICH HAD BEEN INCUBATED FOR 4 DAYS BOTH IN THE PRESENCE AND ABSENCE OF POLYESTER SPONGE, ON THE GRANULATION REACTION AROUND COTTON WOOL PELLETS IN INTACT RECIPIENT RATS

Each result represents the mean of five rats. The average initial body weight of each animal was 155 g. The increases in dry weight of pellets are means and standard errors. The percentage effects, together with the respective P values, compare the effect of the medium and sponge with that of the medium alone (plasma or saline). Other comparisons have been made (for example plasma and sponge with saline and sponge) but none of the resulting differences was statistically significant ($P>0.1$)

Treatment	Initial pellet weight (mg)	Increase in dry weight of pellet (mg)	Effect (%)	P
Saline + sponge	9·2	7·49 ± 0·47	+22	<0·05
Saline	9·2	6·12 ± 0·43		
Plasma + sponge	9·2	6·54 ± 0·71	−11	>0·4
Plasma	9·2	7·35 ± 0·59		
Plasma + sponge + antibiotic	9·2	6·93 ± 0·58	+8	>0·5
Plasma + antibiotic	9·2	6·41 ± 0·67		

Effect of body weight on the extent of the cotton pellet reaction

One additional observation of interest is presented, although it is not immediately relevant to the present experiments. It has been shown by statistical analysis of control results of earlier experiments that the amount of tissue deposited on the cotton wool pellets depends, amongst other factors, on the initial body weight of the animals (Robinson & Robson, unpublished). This has been shown conclusively in an independent experiment in which groups with different mean body weight were used. The results are summarized in Fig. 2, which clearly shows that the dry weight of tissue deposited on pellets of the same size depends on the body weight of the animals in which they are implanted. Analysis shows this correlation to be highly significant ($P<0.001$). It is for this reason that the mean body weights of each group of animals in any one experiment were equalized at the beginning of the experiment.

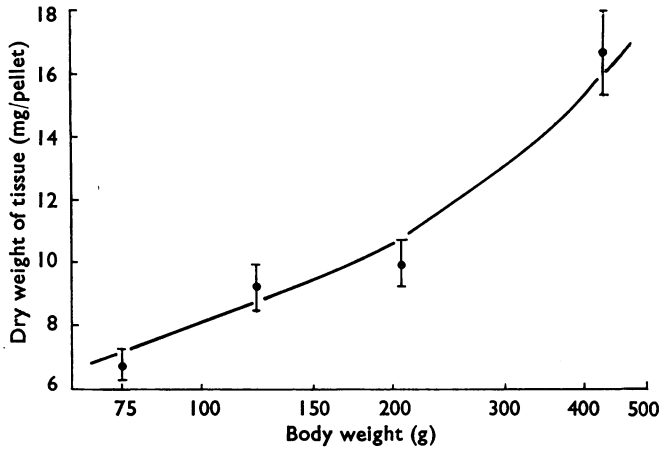

Fig. 2. The effect of body weight (abscissa, log scale) on the amount of tissue deposited on each pellet (ordinate). Points represent means with standard errors. The correlation coefficient for this line is 0·751 with 19 degrees of freedom. This is larger than the tabulated value, at the 0·001% probability level, so correlation is highly significant.

DISCUSSION

Limit to the inflammatory reaction as a possible factor in the anti-inflammatory response

The present experiments show quite clearly that, when irritant substances are injected or implanted, the resulting reduction in the deposition of tissue on implanted cotton pellets is not due to a limit to the possible inflammatory reaction. This has been shown in two types of experiments, namely the implantation of sponges of various sizes, and the implantation of irritant materials in addition to cotton wool pellets.

The hypothesis of Cygielman & Robson (1963), that the body is overwhelmed by the demand on its resources and is then unable to provide sufficient macrophages

to cope with the situation, might well explain what happens in the case of bacterial invasion. However, when irritation is produced by the injection or implantation of irritant substances, for example charcoal or sponges, it merely produces a local reaction, dependent essentially on the degree of local irritation.

The local production of an anti-inflammatory substance

In the present work it has been shown that the apparent anti-inflammatory effect of irritant materials is most probably due to an anti-inflammatory substance produced at the site of inflammation (the site of implantation of the irritant material), which is absorbed into the circulation, and which then acts in other parts of the body to reduce the inflammatory reaction. This hypothesis was first put forward by Laden *et al.* (1958) in explanation of their results. They had shown that oedema induced by injection of an irritant substance into the pleural cavity of the rat could be reduced by injection of a counter-irritant substance into the knee joint, and that this effect persisted in adrenalectomized and hypophysectomized animals.

Di Pasquale & Girerd (1961) and Di Pasquale, Girerd, Beach & Steinetz (1963) have adopted a similar point of view. Using freeze dried exudates from croton oil-induced granuloma pouches, they have shown that, when the reconstituted exudate is injected into other rats, it inhibits the formation of cotton pellet granulomata. Our present findings agree with these results.

We have also obtained some evidence that the anti-inflammatory effect is not due to a steroid. Firstly all experiments were performed on animals which had been adrenalectomized 4 days before the collection of exudate, and, since the half life of hydrocortisone and cortisone are (at least in man) 110 and 30 min respectively (Peterson, 1959), it is unlikely that any appreciable amount will remain in the body when the exudate is collected. Secondly, when steroids are injected into rats there is a significant involution of the thymus, in addition to the reduction of tissue deposited on cotton wool pellets. Following the injection of sponge exudate, no such thymic involution was apparent, nor was there any reduction in the rate of increase of body weight, as would be seen in steroid therapy.

Di Pasquale and his co-workers came to similar conclusions and, in addition, have shown by fluorimetric analysis of chloroform-extracts of the exudate, that only 1.3 μg of corticosterone equivalents are present in each gram of dried exudate, insufficient to account for the anti-inflammatory effects it produced. They also suggest that the anti-inflammatory substance must have a different mode of action to a steroid, on the grounds that, when it is administered together with a steroid, the individual effects do not summate, but have a synergistic action.

It could be argued that the effects observed in our experiments are simply the result of injection of an irritant material contained in the exudate. Certainly in Di Pasquale's experiments no attempt was made to remove the croton oil. However the total amount injected over 9 days is less than 0.5 mg, that is considerably less than the 20 mg/day, shown by Cygielman & Robson (1963) to be necessary for the production of a modest anti-inflammatory effect (only 20% reduction).

In our experiments, although an irritant material (the polyester sponge) was used to produce the inflammatory reaction, it did not form part of the resultant exudate;

nevertheless the possibility cannot be excluded that either an active or irritant material was extracted from the sponge. However, in the single experiment in which sponge was incubated *in vitro* with either saline or plasma, injection of the resulting fluid into recipient rats (in which cotton pellets had been implanted) produced no significant reduction in the inflammatory reaction as compared with the effect of saline or plasma respectively. This not only excludes the possibility that an irritant substance is extracted from the sponge, but also that an active anti-inflammatory substance is extracted from it, and that the plasma on contact with sponge is broken down in some way to produce an active substance. The possibility of reinjection of an irritant substance would thus seem to be excluded, and this conclusion is supported by the absence of any obvious signs of irritation at the injection sites of both sponge exudate and plasma which has been incubated with sponge.

Evidence for the absence of an anti-inflammatory substance in plasma from animals without implanted sponges

We have so far assessed the activity of anti-inflammatory materials (for example the exudate obtained from sponges) by comparing the effect produced in rats with that in controls injected with saline. However, if an anti-inflammatory substance was normally present in the plasma of adrenalectomized animals (without implanted sponges), it could readily account for the activity of the exudate obtained from implanted sponges. Moreover, since the protein content of the exudate is only two-thirds that of plasma, so that in this respect the exudate resembles dilute plasma, it might be fairer to compare the activity of the exudate with such plasma. In the three experiments in which these comparisons were made (Table 4*a, b* and *c*) the sponge exudate always showed a highly significant effect whether compared with saline- or plasma-treated control groups (in each instance, $P<0.001$).

Furthermore, the effect of the plasma was never statistically different from that of saline, although in all three experiments (Table 4*a, b* and *c*) slight but nonsignificant anti-inflammatory effects were observed. The probable explanation of this is that, when an animal is adrenalectomized, an inflammatory site is automatically created at the site of operation, and this may be a source of anti-inflammatory substance. This explanation should be taken into account in assessing the significance of the results.

Evidence for the presence of an anti-inflammatory substance in plasma from animals with implanted sponges

Plasma obtained from adrenalectomized animals in which sponges have been implanted produces a statistically significant anti-inflammatory effect when compared with that from saline-treated controls. For injections into adrenalectomized recipients, $P<0.001$ (combined value; Table 4, *b* and *c*). However if comparison is made with plasma from animals not implanted with sponges, then (due to the slight reduction caused by this plasma) the effect is no longer significant (combined value, $P>0.05$). Because of this it is difficult to be certain that the anti-inflammatory substance is present in the blood.

Di Pasquale and his colleagues have been quite unable to demonstrate any activity in serum obtained from animals in which pouches have been produced, and this, together with the fact that the anti-inflammatory activity of the exudate did not alter with the time after the formation of the pouch, led them to suggest that the anti-inflammatory substance was of purely local production, and had no vascular derivation. They do not consider the possibility that the substance might find its way into the blood, after being produced at the inflammatory site.

It seems likely that the anti-inflammatory substance we have demonstrated is produced locally. Moreover the anti-inflammatory effects observed at distant sites suggest that the locally-produced substance must travel in the blood. This is supported by the fact that there is good, though not conclusive, evidence that plasma possesses some activity. The discrepancy between our results and those of Di Pasquale probably lies in the nature of the inflammatory site. After only 4 days the sponges are not surrounded by a distinct capsule, such as would be present in croton oil-induced pouches after 7 days. The absence of a capsule would in our experiments allow an active substance produced at the inflammatory site to enter the blood stream more readily; and it is quite possible that in the early stages of croton oil pouch formation similar activity would be detectable in the plasma.

Clinical implications

It is very interesting to note that Highton (1963) has shown that daily injections into rats of 2 ml. of serum from patients with active rheumatoid arthritis reduces the amount of tissue produced in carrageenin-induced granuloma pouches and reduces the tensile strength of wounds in such animals. Comparison was made both with saline-treated control rats and with animals injected with serum from non-rheumatoid patients, and in both instances the reduction was highly significant.

It is very tempting to suggest, as others have done, that, during the course of the inflammatory reaction in the body, an active anti-inflammatory substance is produced locally which, depending on the nature of the inflammatory site, may or may not appear in the blood. Such a substance may then play a part in the defence reactions of the body by controlling and ultimately terminating the local inflammatory response at the site of inflammation, and possibly also at remote sites.

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