THE INFLAMMATORY RESPONSE TO IMPLANTATION OF COTTON PELLETS IN THE RAT

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The subcutaneous implantation of a cotton pellet in the rat evokes a short-lasting phase of increased capillary permeability lasting some 20 min. after implantation followed by a more sustained phase which occurs after 2·5–3 hr. The early increase is antagonised by lysergic acid diethylamide, reserpine and 5-HT. The peak granuloma weight is reached at 2 days, and then falls rapidly. Antagonism of oedema and granulation tissue by hydrocortisone and reserpine can be demonstrated at 2 days after implantation.

The cotton pellet test was introduced by Meier, Schuler and Desaulles (1950) to study the effect of local and systemic applications of cortisone upon developing granulation tissue. Since then this method has been extensively used for the evaluation of anti-inflammatory agents.

The time interval between implantation of the pellet and removal of the granuloma has varied. Singer and Borman (1956) chose 4 days; Meyer, Stucki and Aulsebrook (1953), 5 days; Finney and Somers (1958), 6 days; Dulin (1955), 7 days and Setnikar, Salvaterra and Temelcou (1959), 8 days.

During attempts to shorten the duration of the test we became interested in the inflammatory events occurring in the first 4 days after implantation of the cotton pellet in the rat, and we now describe the results of experiments on the following aspects of the inflammatory response: (*a*) the increased capillary permeability around the cotton pellet during the first 5 hr. after implantation using the vital dye technique, and (*b*) the wet and dry weight of the granulomas, and the effect of hydrocortisone and reserpine upon them in the 4 day period after implantation. The wet weights of granulomas removed at intervals up to 28 days after implantation are also reported.

Experimental Methods

Female albino wistar rats, 140–180 g. body weight, were used. They were housed at a room temperature of 21°.

One sterile cotton pellet (Johnson & Johnson), weighing from 6 to 10 mg. was implanted subcutaneously in each groin under ether anaesthesia. The pellet was inserted well clear of the skin wound which was then closed with a stainless steel suture clip.

Measurement of Early Changes in Capillary Permeability using Pontamine Sky Blue as Indicator

The pellets were removed from the rat at intervals of from 10 min. to 5 hr. after implantation. Pontamine Sky Blue (0·2 ml. of 2 per cent solution per 100 g. rat) was injected intravenously 15 min. before removal

of the pellets which were then graded for their degree of blueing, by several different observers, against an arbitrary scale ranging from 1 = no blue, up to 6.

Wet and Dry Weights of Granulomas

The two granulomas were removed and were immediately weighed wet in pairs. The dry weights of the paired granulomas were obtained by drying overnight at 60° to constant weight. In the latter instance the initial pellet weight was subtracted from the final dry weight.

Drugs

Reserpine was dissolved in 1 per cent acetic acid and was injected intraperitoneally in 3 doses of 2 mg./kg., over a period of 5 days before the test, the final injection being 2 hr. before implantation of the pellets. Lysergic acid diethylamide (LSD) and 5-hydroxytryptamine (5-HT) were injected intravenously 30 min. before implantation of the cotton pellets. Hydrocortisone acetate was made up as a saline suspension, one injection of 100 mg./kg. being given intraperitoneally at the time of implantation.

All intravenous injections were made into the tail vein.

RESULTS

Increased Capillary Permeability Around the Pellet during the First 5 hr. after Implantation

There was an initial increase in capillary permeability during the first 20 min. after implantation, demonstrated by the blueing of the cotton pellets. This was followed by a phase of impermeability, during which the pellets remained white, which lasted until 2–2·5 hr. after implantation, when a further increase in capillary permeability became evident (Table I).

TABLE I

THE INCREASE IN CAPILLARY PERMEABILITY AROUND A COTTON PELLET DURING THE FIRST 5 HR. AFTER IMPLANTATION

Time of removal of pellets	No. of pellets	Degree of blueing ± s.e.
10 min.	54	4·67 ± 0·082
15 ,,	12	5·88 ± 0·135
20 ,,	42	4·45 ± 0·102
30 ,,	6	2·17 ± 0·423
40 ,,	6	1·17 ± 0·112
50 ,,	6	1·17 ± 0·112
60 ,,	6	1·58 ± 0·288
1·5 hr.	6	1·50 ± 0·230
2·0 ,,	12	1·54 ± 0·199
2·5 ,,	6	4·33 ± 0·225
3·0 ,,	6	3·58 ± 0·557
4·0 ,,	12	3·87 ± 0·211
5·0 ,,	6	3·83 ± 0·271

The early blueing was significantly reduced by treatment with LSD and reserpine (Table II), and was abolished by 5-HT in suitable doses (Table III). It is interesting to note that the inhibitory effect of LSD and reserpine was greater at 10 min. than at 20 min.

Granuloma Weights

The paired wet granulomas reached a maximum weight by the 2nd day after implantation. They then decreased in weight until day 4 when there was a levelling off with further falls in weight at day 6 and day 14. The granuloma weight then remained at the same level until day 28 (Table IV).

Hydrocortisone acetate (100 mg./kg.) significantly reduced both wet and dry weights of 1–4 day granulomas (Figs. 1 and 2) and reserpine pre-

TABLE II

THE EFFECT OF LYSERGIC ACID DIETHYLAMIDE AND OF RESERPINE UPON EARLY BLUEING OF AN IMPLANTED COTTON PELLET

Time of removal of pellets	Treatment	No. of pellets	Degree of blueing ± s.e.	P
10 min.	Controls	12	4·89 ± 0·127	
10 ,,	LSD 0·5 mg./kg.	12	2·77 ± 0·161	<·001
20 ,,	Controls	12	4·44 ± 0·195	
20 ,,	LSD 0·5 mg./kg.	12	3·44 ± 0·155	<·001
10 min.	Controls	12	4·04 ± 0·211	
10 ,,	Reserpine	12	1·94 ± 0·134	<·001
20 ,,	Controls	12	4·60 ± 0·139	
20 ,,	Reserpine	12	3·00 ± 0·399	<·01

treatment likewise significantly reduced these weights. The granuloma weights (mg. ± s.e.) for groups of 10 rats at 2 days being: no treatment: 397·19 ± 16·91 (wet) 59·79 ± 5·15 (dry); reserpine: 217·65 ± 5·94* (wet) 19·37 ± 1·84* (dry) *P<·001.

DISCUSSION

From the results of the tests using Pontamine Sky Blue it is evident that in the first 5 hr. after implantation of a cotton pellet there is an inflammatory response consisting of 2 phases of increased capillary permeability,

TABLE III

THE EFFECT OF 5-HT UPON EARLY BLUEING OF AN IMPLANTED COTTON PELLET

Time of removal of pellets	Treatment	No. of pellets	Degree of blueing ± s.e.	P
10 min.	None	12	4·62 ± 0·142	
10 ,,	5-HT 20 mg./kg.	12	1·10 ± 0·062	<·001
10 ,,	5-HT 10 mg./kg.	12	1·37 ± 0·121	<·001
10 ,,	5-HT 5 mg./kg.	12	1·25 ± 0·087	<·001
15 ,,	None	12	5·88 ± 0·135	
15 ,,	5-HT 5 mg./kg.	6	2·42 ± 0·146	<·001
15 ,,	5-HT 2·5 mg./kg.	6	4·08 ± 0·224	<·001
15 ,,	5-HT 1·0 mg./kg.	6	4·67 ± 0·238	<·001

an initial one lasting for about 20 min. and a delayed phase appearing after a lapse of 2·5 hr. In this respect the early inflammatory response to insertion of a cotton pellet closely resembles that caused by other procedures such as mild burning of guinea-pig skin (Sevitt, 1958; Wilhelm and Mason, 1958), bacterial inoculations and exposure of skin to ultraviolet light (Miles and Wilhelm, 1961). Whereas the initial oedema may be due partly to the injury resulting from implantation, and partly to the

irritant effects of the cotton itself, the delayed oedema is most probably a response to the cotton pellet. The duration of the initial increase in capillary permeability is similar to that seen after the intradermal injection of histamine, 48/80 and leukotaxine in the guinea-pig (Miles and Miles,

TABLE IV

WET WEIGHT OF PAIRED GRANULOMAS

Time or removal of pellets (days)	No. of rats	Wet wt. in mg. ± s.e.
1	9	320·10 ± 46·09
2	12	495·30 ± 21·33
3	212	444·40 ± 18·170
4	6	318·70 ± 18·320
5	6	313·20 ± 21·700
6	6	209·10 ± 6·900
7	6	212·70 ± 6·100
10	6	210·20 ± 12·800
14	6	170·10 ± 8·900
17	6	175·80 ± 9·700
21	6	160·00 ± 3·800
28	6	165·90 ± 4·300

1952), and after the administration of 5-HT to the rat (Miles and Wilhelm, 1961). In our experiments the antagonism of the initial oedema by LSD, and by pretreatment with reserpine may mean that 5-HT is involved as a mediator of initial oedema since it is known that LSD antagonises 5-HT-induced oedema of the rat paw (Doepfner and Cerletti 1958), and that

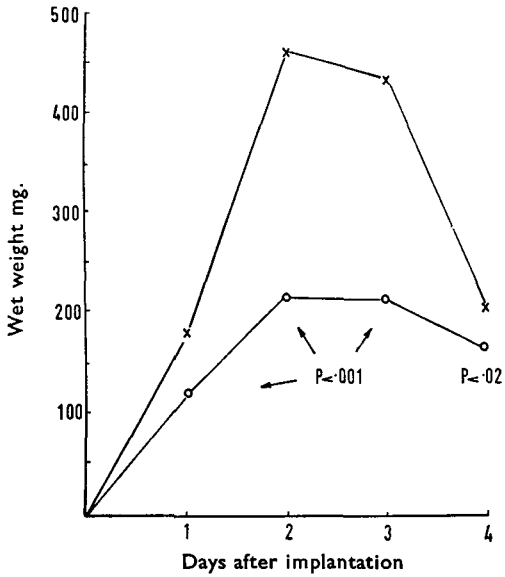

FIG. 1. The effect of hydrocortisone acetate on the development of granulomatous tissue around subcutaneously implanted cotton pellets in the rat. Each point represents the mean wet granuloma plus pellet weight from a group of 5 control and 10 treated rats. Hydrocortisone 100 mg./kg. i.p. was injected at the time of implantation. The probability values refer to the differences between control and treated animals. X—X = Controls. O—O = Hydrocortisone treated.

reserpine will deplete skin and other tissues of its 5-HT content (Parratt and West, 1957).

Rather surprisingly intraperitoneal 5-HT has been shown to inhibit dextran oedema in the rat foot (Georges and Herold, 1957). Similarly Setnikar, Salvaterra and Temelcou (1959) found that iproniazid inhibited formalin-and dextran-induced oedemas, and cotton pellet granulomas in the rat, and suggested that the effect might be due to an anti-inflammatory action of 5-HT. 5-HT also inhibited the formation of fluid in the Selye granuloma pouch (Franchimont, van Cauwenberge and Lecomte, 1961). We have investigated the effect of 5-HT on cotton pellet inflammation and have shown that it antagonised the early increases in vascular permeability, over a very similar dosage-range to that found effective by Georges and Herold (1957).

The mode of action of 5-HT as an anti-inflammatory agent is obscure but may be related to the refractory state of the capillaries which follows

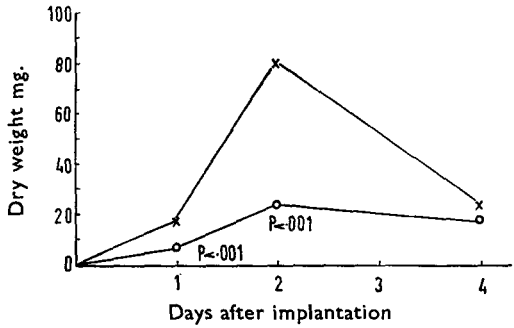

FIG. 2. The effect of hydrocortisone acetate on the development of granulomatous tissue around subcutaneously implanted cotton pellets in the rat. Each point represents the mean weight of dry granulomatous tissue from a group of 5 control and 10 treated rats. Hydrocortisone 100 mg./kg. i.p. was injected at the time of implantation. The probability values refer to the differences between control and treated rats. X—X = Controls. O—O = Hydrocortisone treated.

their stimulation by 5-HT when it is given by intradermal injection (personal observations). A similar refractory state of the capillaries is seen after the intradermal injections of histamine, 48/80 and leukotaxine in the guinea-pig (Miles and Miles, 1952), although it has not yet been shown to follow the administration of 5-HT intravenously. Another possible mechanism of action is via an effect on the adrenals since it has recently been shown that 5-HT stimulates the secretion of hydrocortisone from the perfused adrenal gland of the hypophysectomised dog (Verdesca, Westermann, Crampton, Black, Nedeljkovic and Hilton, 1961). However, Georges (1957) failed to deplete adrenal ascorbic acid in the intact rat using 5-HT.

The rapid increase in wet weight of the cotton pellet granulomas between 0–2 days after implantation may merely represent an extension of the delayed oedema which started at 2·5 hr. after implantation. In this case the equally rapid loss of weight between days 3 and 4 is probably the

result of a loss of fluid from the granuloma. Since the solids contained in such a large amount of fluid in the granuloma at days 2 and 3 will affect the dry weight and therefore the calculated amount of granulation tissue, we determined what proportion of the final dry weight could be accounted for by such solids. Quantities of rat plasma equivalent in weight to that of the 2 day control and hydrocortisone treated granulomas were dried and weighed. The results show that the plasma solids could constitute approximately 1/3 of the control dry weight and approximately half of the hydrocortisone treated dry weight, the remainder being presumably granulation tissue.

The peak weight at day 2 was inhibited by hydrocortisone given in one injection at the time of implantation, and we have subsequently shown that two daily doses (5 mg./kg.) are equally effective at 2 days. The reduction of granulation tissue by hydrocortisone was also apparent visually since granulomas from the treated rats were very thin walled and white compared to the large, hyperaemic granulomas of the controls. It may be possible, therefore, to use a cotton pellet test extending over 2 or 3 days only, for the screening of anti-inflammatory agents.

The antagonism of 2 day old granulomas by reserpine confirms the report recently made by Bhatt and Sanyal (1963) concerning the effect of reserpine on week-old granulomas, and would suggest that the role of 5-HT in the rat is not confined to the mediation of acute inflammatory responses.

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