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# Effect of body weight changes on the formation of cotton pellet-induced granuloma

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Cotton pellet-induced granuloma formation in intact animals is intimately dependent upon body growth and general anabolic processes. Deposition of connective tissue around the pellet continues up to (and probably beyond) the observation period of 90 days after implantation. Anorexic or catabolic agents, or both, or restricted food intake markedly impairs granuloma formation as a result of an impaired body growth. By expressing the amount of granuloma formed in mg/100 g body weight, the true anti-granulomatous properties of a drug can be easily dissociated from those attributable to an impaired body growth. The mechanism by which impairment in body growth affects granuloma formation is discussed.

IT has been reported that the subcutaneous administration of substances which cause marked irritation at the site of injection significantly inhibits the formation of the cotton pellet-induced granuloma (Cygielman & Robson, 1963). These authors conclude that this test is not suitable for the investigation of subcutaneously administered irritating substances. However, this is not the only factor limiting the use of this method. We have repeatedly observed reduced granulomas following the administration of anorexic or catabolic agents or both, or by restricting food intake. To generalise, any appreciable impairment in the normal growth of the animal appears to interfere to a greater or lesser extent with granuloma formation.

In a variety of biological tests, it is common to express the increase or decrease in the weight of certain organs as a percentage of body weight. The application of this conversion factor to the cotton pellet test (mg of granuloma/100 g body weight) makes it possible to dissociate the anti-granulomatous properties of a substance from those attributable to an impairment in body growth.

The object of this paper is to present evidence which indicates the close relationship between body growth and granuloma formation and also to justify the use of the conversion factor.

## Experimental

### MATERIALS AND METHODS

Male Wistar rats, 160 $\pm$ 10 g, were used. Under light ether anaesthesia, one cotton pellet was introduced subcutaneously on each side of the abdomen through a mid-line incision. The pellets were cut from dental cotton rolls (No. 1 Johnson and Johnson) and paired to a combined dry weight of 70 $\pm$ 1 mg. Before implantation the pellets were individually soaked in 0·5% antibiotic (Combiotic, Pfizer) solution. At the end of the test period, the animals were killed by carbon dioxide asphyxiation. The pellets with the surrounding granuloma were removed, dried to a constant weight at 50° and weighed. The amount in excess of 70 mg represents the weight of the granuloma.

From Warner-Lambert Research Institute, Morris Plains, New Jersey.

*Effect of food restriction on granuloma formation.* The rats were individually caged and divided into 6 groups of 8 animals each. Group 1 received ground food *ad libitum*, the other groups received a daily amount of ground food equivalent to 10, 8, 6, 4 and 2% of their original body weight. Food restriction was started on the same day as cotton pellet implantation. The animals were killed 10 days later, and the weights of the granulomas were determined.

*Effect of body growth on granuloma formation.* After cotton pellet implantation, groups of 8 animals were killed at various time intervals up to 90 days, and the weights of the granulomas determined.

*Effect of cortisone acetate on granuloma formation.* The rats were divided into 5 groups of 14 animals each. Group 1 served as control, whereas the others received varying subcutaneous doses of cortisone acetate. Treatment started on the same day as pellet implantation. The animals were killed 10 days later and the weights of the granulomas were determined.

## Results

*Effect of food restriction on granuloma formation.* The data are shown in Table 1. Compared with controls fed *ad libitum*, a reduced rate of

TABLE 1. EFFECT OF FOOD INTAKE RESTRICTION ON GRANULOMA FORMATION

| Amount of food offered as % original body weight | Average body weight in g | | Average body weight changes in g | Average weight of granuloma in mg | | | |
|---|---|---|---|---|---|---|---|
| | Initial | Final | | Absolute values | P | As % body weight | P |
| *Ad libitum* (8)* | 166 | 240 | +74 | 97·0 ± 4·6** | — | 40·4 ± 2·8** | — |
| 10 (8)* | 166 | 211 | −45 | 88·0 ± 4·6 | N.S. | 41·7 ± 2·0 | N.S. |
| 8 (8)* | 169 | 203 | −34 | 82·0 ± 3·4 | <0·025 | 40·4 ± 1·8 | N.S. |
| 6 (8)* | 165 | 165 | 0 | 71·0 ± 2·6 | <0·001 | 43·0 ± 1·6 | N.S. |
| 4 (8)* | 166 | 135 | −31 | 60·0 ± 2·9 | <0·001 | 44·4 ± 2·3 | N.S. |
| 2 (8)* | 169 | 104 | −65 | 42·0 ± 3·3 | <0·001 | 40·4 ± 2·5 | N.S. |

* Number of animals.
** Standard error.

gain to a severe loss from original body weight occurred as the amount of food made available to the animals was proportionally decreased. If the weight of the granuloma was expressed as an absolute value, a parallel proportional decrease in the weight of the granuloma was observed. However, when the weight of the granuloma was expressed as mg/100 g body weight, no significant difference could be detected between the various groups.

*Effect of body growth on granuloma formation.* The results are shown in Fig. 1. When the weight of the granuloma is expressed as absolute value, it is apparent from the slope of the two curves that the growth of the granuloma exceeds that of the body up to 30–36 days after implantation and parallels the latter thereafter. This phenomenon is more obvious when the weight of the granuloma is expressed as mg/100 g body weight.

*Effect of cortisone acetate on granuloma formation.* The results are shown in Table 2. Compared with untreated animals, a reduced rate of

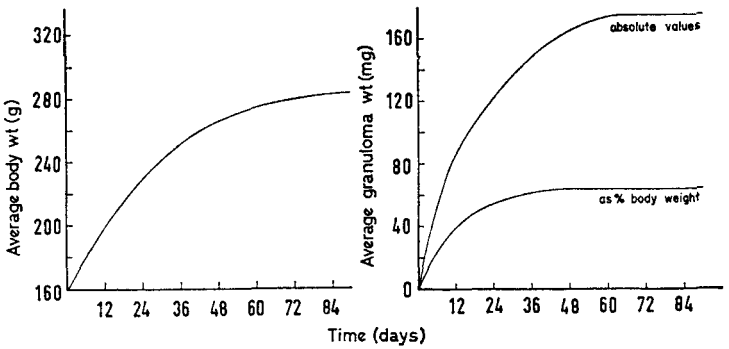


FIG. 1. Effect of body growth on granuloma formation.

TABLE 2. EFFECT OF CORTISONE ACETATE ADMINISTRATION ON GRANULOMA FORMATION

| Treatment | Daily dose mg/kg | Route | Average body weight in g | | Average body weight changes in g | Average weight of granuloma in mg | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | | Absolute values | P | As % body weight | P |
| None (14)* | — | — | 163 | 224 | +61 | 98·0 ± 4·3** | — | 43·7 ± 1·8** | — |
| Cortisone Ac. (14)* | 5 | s.c. | 155 | 210 | +55 | 91·0 ± 6·1 | N.S. | 43·3 ± 2·5 | N.S. |
| Cortisone Ac. (14)* | 10 | s.c. | 159 | 194 | +35 | 77·0 ± 4·7 | <0·005 | 39·7 ± 2·2 | N.S. |
| Cortisone Ac. (14)* | 20 | s.c. | 158 | 175 | +17 | 60·0 ± 3·4 | <0·001 | 34·3 ± 1·7 | <0·001 |
| Cortisone Ac. (14)* | 40 | s.c. | 161 | 156 | −5 | 49·0 ± 1·8 | <0·001 | 31·4 ± 1·1 | <0·001 |

* Number of animals.
** Standard error.

gain to a slight loss from the original body weight occurred in proportion to the amount of subcutaneously administered cortisone acetate. The significant reduction in granuloma formation following the 10 mg/kg dose was probably due to an impairment in body growth since no difference was detectable when the weight of the granuloma was expressed in mg/100 g body weight. At the 20 and 40 mg/kg doses, the reduction in granuloma formation was over and above that which could be attributed to an impairment in body growth. Significant reduction could, therefore, be detected even when the weight of the granuloma was expressed in mg/100 g body weight.

## Discussion

The cotton pellet-induced granuloma is a widely used method for assessing anti-inflammatory activity. It seemed, therefore, worthwhile to study one of the aspects, which although not generally taken into consideration, may influence the interpretation of the results.

Our experiments indicate that granuloma formation in intact animals is intimately dependent upon body growth and general anabolic processes. In fact, any impairment in body growth, whether obtained by restricting food intake or by drug administration, considerably slows down the rate of granuloma formation.

The inflammatory reaction produced by the foreign body probably stimulates the rapid deposition of connective tissue around the pellet during the first few days after implantation (Eichhorn & Sniffen, 1964). It is very unlikely that it could be responsible for the further and relatively slower deposition which continues up to and probably beyond 90 days. This latter finding is in disagreement with the observation (Penn & Ashford, 1963) that granuloma formation reaches its peak on the second day and declines thereafter, although the different site of implantation (groin) could have been responsible for such a phenomenon.

Preliminary experiments (unpublished) indicate a decreased effectiveness of anti-inflammatory compounds when the administration of such compounds is proportionally delayed after the time of cotton pellet implantation. It appears that the antigranulomatous effect of cortisone acetate and other anti-inflammatory compounds is twofold: (*a*) direct inhibition of the inflammatory process and (*b*) indirect inhibition caused by the reduced body weight gain or body weight loss. To generalise, if body weight change is not considered, a falsely high anti-inflammatory effect may be attributed to compounds under test.

The decrease in the rate of granuloma formation attributable to an impairment in body growth can be easily dissociated from that produced by drug administration by expressing the amount of granuloma formed in mg/100 g body weight. Our data clearly show that by using this conversion factor, the granuloma inhibitory effect of the lower doses of cortisone acetate are attributable to its well-known anorexic or catabolic activities, or both, which results in a reduced rate of body growth. Only at the higher doses did this compound show an inhibitory effect over and above that which could be attributed to an impairment in body growth.

The mechanism by which restriction in food intake suppresses granuloma formation is not known. Since intact rats were used, an adrenocortical stimulation in response to stress from starvation cannot be excluded. Restricted diets gave responses comparable to high doses of cortisone acetate only when absolute values were used but not when the conversion factor was utilised. If adrenocortical stimulation alone was responsible for this effect, significant reduction should have been evident even when granuloma weight was expressed in mg/100 g body weight, as was seen with the higher doses of cortisone acetate. Another possibility is that inadequate nutrition may directly impair growth and division of fibroblasts in the proliferating granuloma.

## References

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