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# Anti-cancer action of retinoids

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**Summary.** The anti-cancer action of retinyl acetate (Vitamin-A acetate, VAA), chosen as a representative retinoid substance, is attributed to its power to exercise immunopotentiation, though other possibilities are considered. The reasons for forming this opinion were: (1) chronic administration of VAA brought about enlargement of the thymus and peripheral lymph nodes; (2) the administration of VAA curtailed the life of skin allografts though (3) its action could be reversed by the concomitant administration of immunosuppressive agents.

## INTRODUCTION

'Retinoids' a coinage of M. B. Sporn's—comprise retinol (Vitamin-A alcohol) and its natural or synthetic derivatives, among them retinal, retinoic acid and a variety of ethers and esters derived from the parent compound. Although they vary greatly in toxicity and duration of storage, many retinoids have Vitamin-A activity. It is, however, their anti-cancer action that has aroused most attention in recent years (Chu & Malmgren, 1965; Bollag, 1972; Moon, Grubbs & Sporn, 1976; Bjelke, 1975; Basu, Donaldson, Jenner, Williams & Sakula, 1976; Sporn, 1977; Sporn & Newton, 1979). These considerations prompted us to try to find out how one such retinoid, retinyl acetate (Vitamin-A acetate, VAA)) exercises the anti-cancer action already reported upon by Moon *et al.* (1976), and Medawar, Hunt & Mertin (1979). In this paper we consider the matter under the three headings that seemed most likely to be relevant to a solution of the problem: (1) the effect of VAA on lymphoid tissues in mice; (2) immunopotentiation by VAA; (3) reversibility of the action of VAA by immunosuppressive agents.

The mice used in these experiments were members of highly inbred domestic sublines of strain CBA, C3H and C57Bl/10 maintained upon a conventional pelleted diet, *ad lib*: 'SAD'*, with or without supplementary VAA in the form of stable gelatinized beadlets kindly supplied by Roche Products Ltd, Welwyn Garden City.

## METHODS AND RESULTS

*The effect of VAA on lymphoid tissue*

*Lymphoid hypertrophy.* Female CBA mice received a daily supplement of 250 mg/kg VAA in SAD meal for 21 days. AT the same time age-matched controls received SAD (pelleted form). These mice were used to ascertain: (1) differences in body weights and the relative weights of the principal lymphoid organs; (2) histological differences, and (3) proportion of $\theta$-positive cells.

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* SAD is Spratts Laboratory Diet 1, Spillers Ltd, Newmarket, Suffolk.

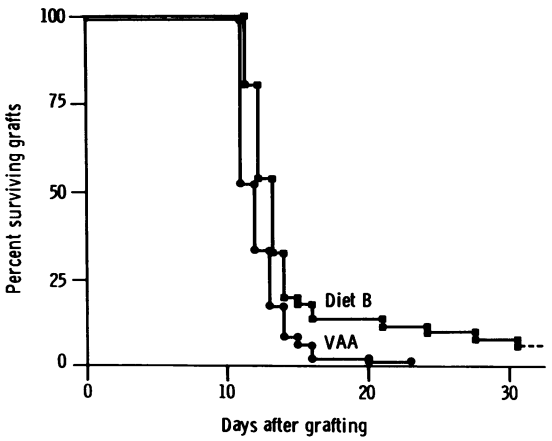


**Figure 1.** Life tables illustrating the survival of female C3H skin allografts grafted on to female CBA mice. The upper curve relates to the mice reared upon the conventional (SAD) diet, the lower curve represents mice which received in addition gelatinized beadlets containing 250 mg/kg of VAA from the day of grafting. Comparison of the two life tables by the log rank method (see text) shows that they differ significantly ($P < 0.025$).

The body weights and the relative weights of the principal lymphoid organs were as shown in Table 1; analysis of the figures showed that there had been significant relative hypertrophy of the peripheral lymphoid organs ($P \ll 0.001$), accompanied by a relative enlargement of the paracortical areas—a finding which suggested that the lymph node hypertrophy might have been secondary to the thymic hypertrophy ($P < 0.001$); there was in addition an absolute but not proportional increase of $\theta$-positive cells in the peripheral lymph nodes of the mice receiving supplementary VAA (Table 2).

*Immunopotentiation*

Immunopotentiation by retinol (Dresser, 1968) and other retinoids, especially retinoic acid, is already well known (Bollag, 1972; Floersheim & Bollag, 1972; Dennert & Lotan, 1978). Although it would be begging the question to attribute the anti-cancer action of VAA (Medawar *et al.* 1979) to immunopotentiation, experiments on the survival times of skin allografts left no doubt that VAA shares this property with other retinoids: the administration to female CBA mice of 250 mg/kg VAA from day of grafting reduced the median survival time (MST) of female C3H skin allografts from 12·5 to 11·0 days; using a more sensitive system (skin grafts from male to female C57 mice), the administration of VAA from 14 days before grafting reduced the MST from 27·5 to 16·5 days; but when VAA was administered from the day of grafting the reduction was only from 27·5 to 20·0 days, with a corresponding reduction in the survival time of second-set graftings (from 11·5 to 10·0 days).

In one experiment (Fig. 2a) in which C57Bl/10 female mice were treated with 250 mg/kg VAA from their day of grafting with male C57Bl/10 skin an accelerated graft rejection was seen. However, when these mice were fed a normal diet for 2 weeks then regrafted

**Table 1.** Bodyweights (g) and relative weights (mg/g body wt) of lymphoid organs of CBA mice reared on conventional diet (SAD) or SAD supplemented by Vitamin A acetate (VAA). Each entry a mean of 10 determinations ± standard sampling error of mean

| | SAD | SAD+VAA | Effect of VAA* |
|---|---|---|---|
| Bodyweight | 20·2±0·31 | 18·5±0·34 | Slight reduction of weight ($P < 0.02$) |
| Relative spleen weights | 3·584±0·143 | 3·718±0·141 | No effect |
| Relative lymph node† weights | 0·877±0·03 | 1·718±0·06 | Significant enlargement ($P < 0.001$) |
| Thymus relative weights | 2·684±0·11 | 3·507±0·11 | Significant enlargement ($P < 0.001$) |

* $P$ represents probability that VAA and SAD readings can be construed as random samples from the same normally distributed population.

† Pooled axillary brachial and inguinal nodes.

**Table 2.** Number of cells in lymphnodes of mice fed conventional diet (SAD) or supplemented by Vitamin-A acetate (VAA). The increase in the number of theta-positive cells of the latter was due to the overall increase in cell population not a higher proportion of T cells

| Total No. of cells (VAA) | | Total No. of cells (SAD) | |
|---|---|---|---|
| Theta positive % | Others % | Theta positive % | Others % |
| 67 | 33 | 71 | 29 |

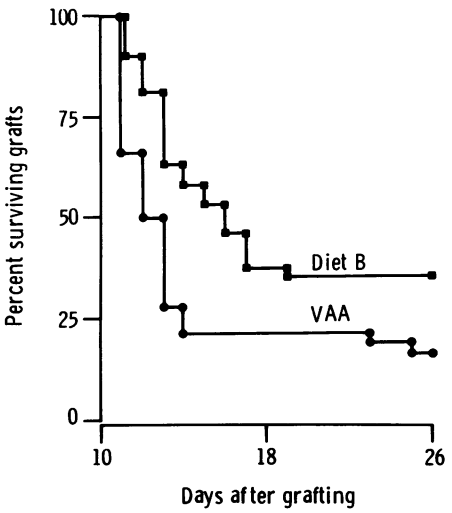


**Figure 2a.** Life tables of male C57 skin grafts upon female C57 mice. The upper curve labelled 'diet B' represents survival in mice on conventional (SAD) diet; the lower curve (VAA) illustrates the acceleration of breakdown in mice fed in addition upon 250 mg/kg VAA from the day of grafting.

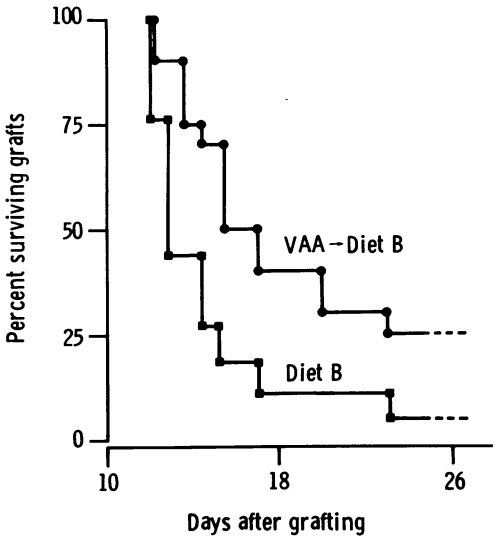


**Figure 2b.** Second set responses towards male skin grafts of the mice illustrated in Fig. 2a. The lower curve illustrates the mice maintained upon diet SAD throughout; these show a normal second-set response. The upper curve is the life table of skin grafts on mice which, having received supplementary VAA, were transferred 14 days before the second graft to diet SAD. As explained in the text the mice failed to give any normal second-set response.

with male skin, they were unable to make the normal second-set reaction that mice fed on SAD throughout the experiment exhibited (Fig. 2b). This anomalous consequence of switching diets was also observed in an experiment with C3H allografts. The mechanism of 'enhancement' is not yet sufficiently well understood to interpret this phenomenon.

*Antagonism by immunosuppressive agents*

If VAA acts through immunopotentiation its effect should be at least partially annulled by the administration of immunosuppressive agents. Medawar *et al.* (1979) have already shown that linoleic acid, which was already known to have immunosuppressive properties (Mertin, 1976; Mertin & Hunt, 1976), can diminish the heightened resistance against methylcholanthrene-induced tumours conferred upon CBA mice by VAA. In our present experiments, illustrated by Fig. 3, it can be seen that the administration of antithymocyte serum raised by the method advocated by Levey & Medawar (1966) can oppose the action of VAA in curtailing the life of allografts of skin: analysis of the graft mortality curves illustrated in Fig. 3 by the log rank method (Peto, Pike, Armitage, Breslow, Cox,

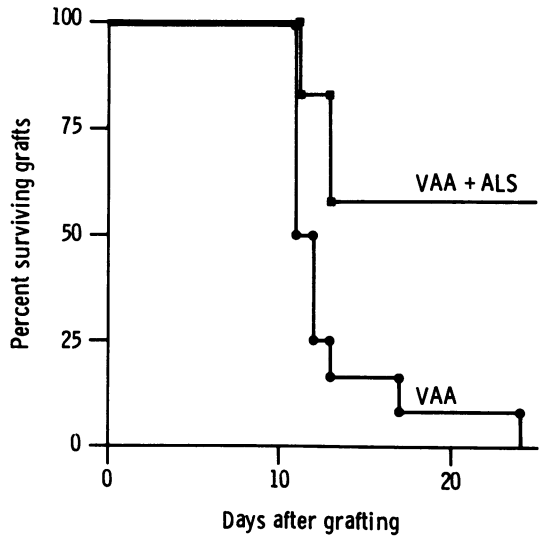


**Figure 3.** Partial annulment of the effects of VAA by anti-lymphocyte serum: all mice were fed throughout the experiment on supplementary VAA. In addition (upper curve) some mice received 0·2 ml anti-thymocyte serum s.c. on days 1, 3 and 5 after grafting. The annulment of the effect of VAA is self-evident.

Howard, Mantel, McPherson, Peto & Smith (1976); Peto, Pike, Armitage, Breslow, Peto & Smith (1977) show the effect to be, in a statistical sense, highly significant.

## DISCUSSION

Although there is clearly a *prima facie* case for thinking that retinyl acetate, and possibly other retinoids, act through the immunopotentiation they undoubtedly exercise, Stutman's (1978) criticisms of any simplistic interpretation of anti-tumour immunity obliges us to consider other possibilites: it may be, for example, that retinoids activate or promote the induction of 'natural killer cells' as the work of Dennert & Lotan (1978) seems to suggest. As to the well-attested power of retinoids to delay the onset and rate of formation of tumours raised by oncogenic hydrocarbons such as 3-methylcholanthrene, the work of Hill & Shih (1974) raises the possibility that retinoids interfere with the metabolic transformation of such hydrocarbons into their oncogenically active forms. This interpretation does not appear to us to be a likely one for the following reasons: (a) in many experiments retinoids have been active when given *after* administration of hydrocarbons and (b) the administration of retinoids has been shown to diminish susceptibility to tumours raised by non-hydrocarbon oncogens, among them methylhydrazine and methylnitrosourea (Sporn, Squire, Brown, Smith, Wenk & Springer, 1977).

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