Vitamin A–Induced Synthesis of Alkaline Phosphatase

Abstract. *Incorporation of radioactive leucine into electrophoretically separated proteins from mouse tail epidermis indicates that synthesis of alkaline phosphatase is stimulated by vitamin A. It is suggested that some of the diverse effects of vitamin A may be the result of alkaline phosphatase induction.*

Regular applications of vitamin A to the tail skin of the house mouse (*Mus musculus*) altered epidermal keratinization and also caused strong alkaline phosphatase activity to appear in the peripheral cytoplasm of the basal and prickle cells, the active zone being spatially separated from the normally reactive granular layer. Untreated mice showed a very weak reaction in the basal cells, but the prickle cells were negative (*1*). Similarly stimulated human epidermis showed changes in keratinization, but no increase in alkaline phosphatase (*2*). Other powerful mitotic stimulants failed to increase mouse epidermal alkaline phosphatase (*3*), which suggests that vitamin A probably has a specific effect on this enzyme independent of keratinization.

Vitamin A could influence alkaline phosphatase activity in various possible ways, and the object of this investigation was to determine its mode of action. Thus, it could stimulate synthesis, or it might be a cofactor, cause aggregation of inactive subunits, or reduce enzyme breakdown.

The tails of adult male "to" mice were painted daily for 3 weeks with a solution of water-miscible vitamin A acetate containing 100,000 units per milliliter. Afterwards the tail epidermis was separated by the calcium chloride method (*4*). Pooled samples of skin from groups of ten mice were ground up in 0.9 percent saline and the supernatant was collected for analysis after centrifugation.

Animals were injected intradermally about 2 cm from the base of the tail with 0.025 ml (5 μc) of ^{14}C-leucine daily on 3 consecutive days during the final week of treatment, and were killed 1 day later. Actinomycin D was dissolved in 0.9 percent saline in a dilution of 0.04 mg/ml, and the mice were injected intradermally about 5 cm from the base of the tail with 0.025 ml of this solution on each of the last 4 days. The injection schedule was arranged so that the mice received two doses of actinomycin D before ^{14}C-leucine was administered, and a 6-hour interval between injections of these agents was maintained for the rest of the period.

Protein from the extracts was separated by starch gel electrophoresis (*5*) at 4°C for 16 hours (0.2 volt/cm^2). The gels were developed for alkaline phosphatase by the coupling method (*6*) using sodium naphthyl phosphate and Fast blue RR salt at *p*H 9.5, and afterwards were cut up into rectangles (0.5 × 1 cm) along the migration track for determination of protein nitrogen (*7*) and for radioactivity (*8*). Two alkaline phosphatase bands were obtained from normal mouse serum in barbital-buffered gels at *p*H 9.5, migrating respectively 2 cm (strong band) and 4.4 cm (weak band) toward the anode. Epidermal alkaline phosphatase migrated 1.6 cm in both vitamin A–treated mice (with a strong reaction) and in control samples (with a very weak reaction). Movement was slower at *p*H 8.7, and at *p*H 5.6 in borate buffer both the epidermal and serum bands migrated similarly 0.4 cm toward the cathode after 16 hours at 0.15 volt/cm^2.

The results of ^{14}C-leucine incorporation into total epidermal protein and electrophoretically separated protein in alkaline phosphatase bands are shown in Table 1. The values suggest that the overall incorporation into epidermal proteins is unaffected by vitamin A, while the incorporation into proteins migrating in the alkaline phosphatase band is three to four times greater in vitamin A–treated material. Figure 1 shows that there is a close correspondence between the position of the alkaline phosphatase band and the peak radioactivity.

Table 1. Incorporation of radioactive leucine into total extracted epidermal protein and alkaline phosphatase bands of vitamin A–treated and control animals. Specific activities given as disintegrations per minute (above background) per microgram of protein nitrogen.

Sample	Specific activity
Total epidermal extract (vitamin A treated)	51.89
Total epidermal extract (control)	53.18
Epidermal alkaline phosphatase (vitamin A treated)	16.5
Epidermal alkaline phosphatase (control)	4.8
Serum (vitamin A–treated animals)	2.1
Serum (control)	1.8

Actinomycin D blocks RNA synthesis (*9*), and its effect on ^{14}C-leucine incorporation is seen in Fig. 2. In the dosage used (~ 2 μg/g), actinomycin D caused about a 50 percent reduction in ^{14}C-leucine incorporation into epidermal proteins, including protein in the alkaline phosphatase electrophoretic band, extracted from vitamin A–treated mouse tail epidermis.

The present findings strongly suggest that vitamin A specifically induces the DNA-directed synthesis of alkaline phosphatase. A vitamin A–stimulated

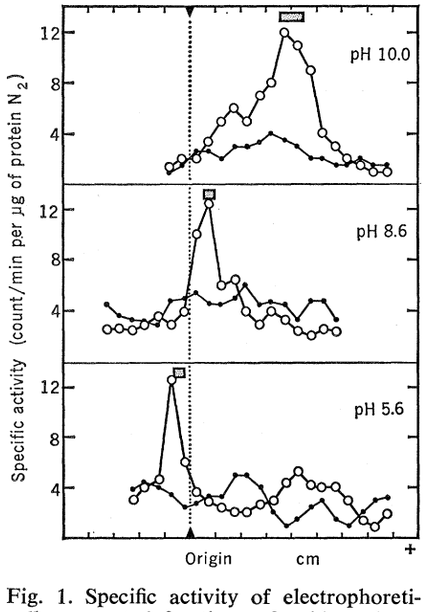

Fig. 1. Specific activity of electrophoretically separated fractions of epidermal extracts. The specific activities are given as counts per minute above background per microgram of protein nitrogen. Open circles, vitamin A; filled circles, control; stippled boxes, alkaline phosphatase.

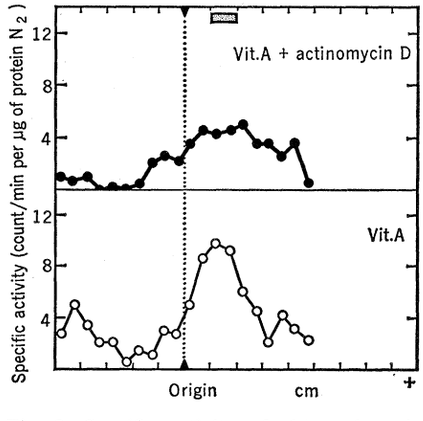

Fig. 2. Specific activity of electrophoretically separated epidermal proteins from vitamin A–treated animals showing the effect of actinomycin D. Stippled box, alkaline phosphatase.

increase in RNA synthesis might therefore be expected in cells which synthesize large amounts of this enzyme (*10*). The functional significance of vitamin A–induced alkaline phosphatase synthesis is uncertain, but some of the effects of vitamin A in bone formation (*11*) and in lipid deposition and transport (*12*) might be mediated through this enzyme, since it is required for phosphate transfer in both types of metabolism (*13*). Alkaline phosphatase is also increased by vitamin A in certain protein-secreting cells having membrane-bound ribosomes (*14*) where it may be more directly concerned with membrane phospholipid metabolism.

Alkaline phosphatase is increased by hypervitaminosis A in tissues other than epidermis, as in the epiphyseal osteoblasts of rats (*15*) and in human blood plasma (*16*), which suggests that in general it may be vitamin A–dependent. In the body it occurs as a variety of genetically independently determined molecular forms (isoenzymes) which are often tissue specific although having similar enzymic activity (*17*). This variation might have evolved fortuitously, but there could be a functional significance if, as seems possible, alkaline phosphatase is in general vitamin A–dependent and if these isoenzymes have different threshold requirements for synthesis. This would explain why, for example, neurones and the blood vessel endothelium are rich in alkaline phosphatase in the presence of relatively low vitamin A concentrations (~ 100 μg/100 ml) (*18*), whereas the epidermal prickle cells require ~ 50,000 μg/100 ml for comparable synthesis to occur. In view of this, it is interesting to note that Fell and Mellanby (*19*) considered that the various tissues probably have different threshold requirements for vitamin A.

P. A. RILEY
R. I. C. SPEARMAN
Department of Chemical Pathology, University College Hospital Medical School, London, W.C.1, England

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20. We thank Dr. K. R. Rees and Dr. A. Jarrett for their interest. Mr. V. K. Asta kindly drew the figures. The vitamin A was a gift from Vitamins Ltd. (Beechams Ltd.). We are grateful to the Medical Research Council for financial support.

1 April 1968

Cystathionine Synthase in Tissue Culture Derived from Human Skin: Enzyme Defect in Homocystinuria

Abstract. *Fibroblasts derived from normal human skin and from cells in amniotic fluid and grown in tissue culture have cystathionine synthase activity. Skin from homocystinuric patients gives rise to fibroblast lines with normal activities of methionine-activating enzyme, but with very low or undetectable cystathionine synthase activity. Thus, the enzyme lesion in homocystinuria is demonstrable in readily available human cells. Neither cystathionine synthase nor methionine-activating enzyme could be detected in intact normal skin.*

Homocystinuria is an inherited metabolic aberration (*1*) characterized by mental retardation, dislocation of the crystalline lens, arachnodactyly, and thromboembolic episodes. Homocystinuric patients have a specific deficiency of cystathionine synthase activity in liver (*2*, *3*) and in brain (*4*). The affected enzyme is in the methionine-to-cysteine pathway. It catalyzes the condensation of homocysteine with serine to form cystathionine.

We have studied this transsulfuration pathway in a variety of mammalian cells grown in tissue culture and have tried to find a readily available source of human cells exhibiting cystathionine synthase activity. Studies of this enzyme in humans have been limited by the need to work with either liver biopsy specimens or material obtained postmortem.

We have determined the cystathionine synthase activity and methionine-activating enzyme [adenosine triphosphate: L-methionine adenosyltransferase, E.C. 2.5.1.6. (*5*)] activity in fibroblasts cultured from small skin biopsies of homocystinuric patients and control subjects. We used cells after 2 to 27 serial subcultivations in monolayer culture.

The results are summarized in Table 1. For controls, cells derived from 21 normal volunteers or patients with diseases other than homocystinuria were studied. The specific activities of cystathionine synthase are expressed relative to the number of cells extracted and to the protein content of the extract. These two measurements correlated well with one another ($r = .93$, $P < .001$). Evaluation of the data did not suggest that a correlation exists between enzyme activities and such variables as the length of time the cells had been stored after harvest, the age or sex of the donor, the presence or absence of disease other than homocystinuria, the number of subcultivations, or the age or density of the cell sheet at the time of harvest. There appeared to be no trend toward either increasing or decreasing enzyme activities during the 20-month course of the study. The specific activities of methionine-activating enzyme and cystathionine synthase appeared to vary independently.

Cell lines were derived from skin biopsies of six patients, each of whom excretes excessive amounts of homocystine in his urine, has dislocated lenses and, in some cases, other of the clinical stigmata of homocystinuria. In their characteristics in culture, the cell lines derived from these homocystinuric patients were indistinguishable from the control lines. The fibroblasts of four of these lines had no detectable cystathionine synthase as measured by a method (*6*) which is about ten times more sensitive than the one used for the control cells. The modified assay permitted chromatographic identification of