**Table 2** Weights of C3H Mice on Control (C) and Antioxidant-containing (A) Diets (Gm)

| | 97 | 125 | 197 | 248 | 310 | 409 | 533 |
|---|---|---|---|---|---|---|---|
| ♂ C | 25.4±1.86 (22–30) n=20 | 27.7±2.62 (23–31) 20 | 31.9±2.84 (29–36) 20 | 32.6±2.64 (29–34) 20 | 30.5±1.73 (26–33) 20 | 30.5±2.12 (26–32) 19 | 29.2±3.04 (26–31) 13 |
| A | 26.0±1.79 (23–29) n=20 | 28.3±2.11 (25–34) 20 | 28.9±2.29 (25–36) 20 | 27.9±2.44 (24–34) 20 | 25.2±2.72 (22–29) 20 | 26.3±2.87 (22–30) 20 | 26.0±3.04 (22–36) 19 |
| ♀ C | 20.2±1.30 (18–23) n=20 | 22.1±1.70 (20–25) 20 | 26.8±2.59 (22–31) 19 | 28.7±3.15 (24–33) 19 | 27.3±3.03 (23–33) 19 | 26.6±3.45 (21–31) 18 | 25.9±2.99 (25–32) 13 |
| A | 20.9±2.27 (16–28) n=20 | 22.3±1.78 (20–28) 20 | 24.4±2.37 (21–29) 20 | 24.1±2.05 (22–28) 20 | 20.6±2.04 (18–25) 20 | 21.0±2.51 (17–25) 20 | 20.3±1.78 (17–24) 18 |

Values are mean, s.d. and range.

inducers—ethoxyquin in these doses causing marked liver enlargement[16]: in Ross's experiments, hepatic enzyme concentrations were found to correlate strongly with further rat life expectancy on various diets[17]; (4) that with the doses used a hypothesis of straightforward "chemical stress", with or without suppression of a predominant tumour, is a feasible cause of longer gross survival. None of these possibilities has been excluded. Experiments with other potent enzyme inducers, such as DDT or barbiturates, seem to be necessary.

The form of the curves, and their response to a mortality incident affecting all groups after between 500 and 600 days, which was probably infective or environmental, would be consistent with a postponement of some predominant age-dependent process by about 15%, rather than with the suppression of one incidental cause of senile mortality. Non-actuarial indices of ageing (pigment deposition, tumour incidence) remain to be examined and will be reported later. We wish, however, to confirm the existence of the effect in a strain previously reported to be unaffected by antioxidants[18].

The Research Group was maintained by the Medical Research Council. We thank Monsanto Ltd for samples of 'Santoquin'.

A. COMFORT
I. YOUHOTSKY-GORE
K. PATHMANATHAN

*MRC Group on Ageing,*
*Department of Zoology,*
*University College London WC1*

Received October 14, 1970.

1 Harman, D., *Gerontologist*, **8**, 13 (1968).
2 Harman, D., *J. Gerontol.*, **23**, 476 (1968).
3 Buu-Hoi, N. P., and Ratsimamanga, A. R., *CR Soc. Biol.*, **153**, 1180 (1959).
4 Harman, D., *J. Gerontol.*, **11**, 298 (1956).
5 Tappel, A. L., *Geriatrics*, **23**, 97 (1968).
6 Dormandy, T. L., *Lancet*, ii, 684 (1969).
7 Marco, G. J., Machlin, L. J., Emery, E., and Gordon, R. S., *Arch. Biochim. Biophys.*, **94**, 115 (1961).
8 Tappel, A. L., and Zalkin, H., *Arch. Biochim. Biophys.*, **80**, 333 (1959).
9 Gerschman, R., *Proc. XXI Intern. Cong. Cienc. Fisiol.*, Buenos Aires 1 (1959).
10 Gerschman, R., Gilbert, D. L., and Caccamise, D., *Amer. J. Physiol.*, **192**, 563 (1958).
11 Taylor, D. W., *J. Physiol.*, **140**, 37 (1958).
12 Gerschman, R., Gilbert, D. L., Nye, S. W., and Fenn, W. O., *Fed. Proc.*, **14**, 56 (1955).
13 Sobel, H., *Aerospace Med.*, **41**, 524 (1970).
14 Machlin, L. J., and Gordon, R. S., *Proc. Soc. Exp. Biol. Med.*, **103**, 659 (1960).
15 Machlin, L. J., *J. Amer. Oil Chem. Soc.*, **40**, 368 (1963).
16 Wilson, R. H., *J. Agric. Food Chem.*, **28**, 375 (1956).
17 Ross, M. H., *J. Nutrit.*, **97**, 565 (1969).
18 Harman, D., *J. Gerontol.*, **12**, 257 (1957).

# Stimulation by Artificial Lighting of Calcium Absorption in Elderly Human Subjects

VITAMIN D, the "sunshine vitamin", is produced in the skin when ultraviolet radiation is absorbed by the pro-vitamin 7-dehydrocholesterol[1]. The endogenous vitamin, together with dietary vitamin D, is then apparently converted in the liver to 25-OH vitamin D, and this active metabolite facilitates the intestinal absorption of calcium and the uptake and release of calcium by the skeleton[2,3].

The amounts of vitamin D usually produced in the intact human skin are unknown. Ultraviolet irradiation of the skin cures childhood rickets[4–7]; in this case endogenous production must be at least equivalent to the minimum curative dietary dose of 200–400 IU daily[8]. Extrapolation of these results to adults is difficult, because their dietary requirement for vitamin D is not established[8]. But there is indirect evidence, summarized by Loomis[9], that the production of vitamin D induced by ultraviolet light is important in the skin.

Ordinary window glass absorbs essentially all radiation of the wavelength necessary for this *in vivo* synthesis—between 275 and 310 nm—of vitamin D[4–7]. Millions of people work behind glass, underground or in the extreme north, travel to and from work in closed vehicles, and venture outdoors only in the early morning or late evening, when ultraviolet radiation is minimal[10]. Incandescent bulbs emit little ultraviolet radiation; the small amount from ordinary fluorescent bulbs is usually absorbed by the fixtures in which they are mounted. We have examined changes in calcium absorption after exposure to 'Vita-Lite' (Duro-Test Corporation, North Bergen, New Jersey), a commercial fluorescent lamp of conventional geometry and loading, designed to duplicate Sun and sky radiation at a colour temperature of 5,500 K (CIE D-5500). About 5% of its total radiant power lies at wavelengths between 290 and 380 nm. Our data suggest that illumination which simulates natural light significantly increases the efficiency of intestinal calcium absorption in people who receive no ultraviolet light from the Sun.

Eighteen white, male residents of the Chelsea Massachusetts Soldiers' Home were selected on the basis of ability to cooperate, age (57–80) and freedom from significant disease. All studies were done with their voluntary consent.

From December 20, 1968, to April 25, 1969, subjects were asked to stay indoors and away from open windows during daylight. Men who normally took multivitamin preparations containing vitamin D were given a substitute lacking this vitamin. Their normal diet contained restricted types of seafoods. They were asked to maintain their habitual intakes

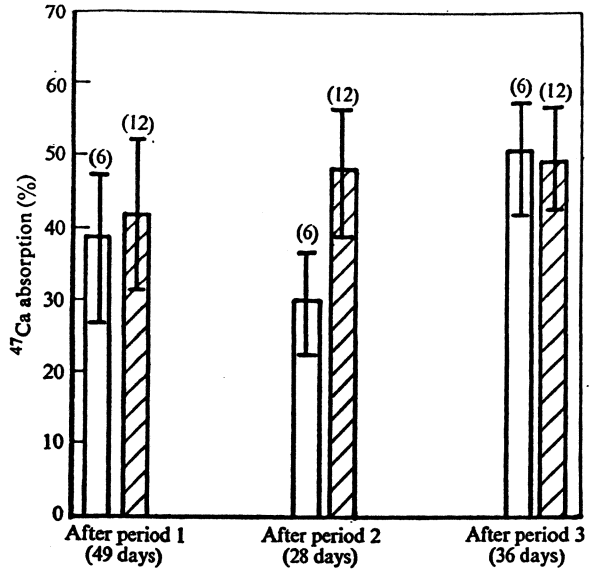


Fig. 1 Absorption of $^{47}$Ca among six control and twelve experimental subjects during the three periods of the study (means ± s.e.). The experimental group was exposed to 'Vita-Lite' during period 2. White columns, control group; hatched columns, experimental group.

of dairy products, which provided an estimated 200 IU or less of vitamin D daily. Their estimated calcium intake throughout the study was 500–1,000 mg daily, the recommended normal intake[11]. Subjects who habitually consumed less than this were given daily calcium supplements.

The study was divided into three periods: period 1 (December 20, 1968–February 7, 1969) was used to allow endogenous vitamin D and 25-OH vitamin D stores to stabilize at new levels consistent with the changes in routine. The time needed to attain this steady state was unknown; 7 weeks were allowed arbitrarily. During this time, subjects were exposed to the low level of ambient lighting (10–50 foot candles of incandescent and/or fluorescent) usually present within the Chelsea Home. At the end of period 1, the ability of each subject to absorb calcium was estimated by measuring the percentage of $^{47}$Ca passed in the faeces within 6 days of ingestion. A complete metabolic research ward was established at the home for all data collection periods. One microcurie of $^{47}CaCl_2$ in 4 fluid ounces of milk was given 2 h before breakfast after an overnight fast. Complete 6 day faecal collections were homogenized, and 1,500 ml. aliquots were counted with appropriate standards to a precision of ±1.5% in an 'Atomium GD-1' detector, using a lower discriminator of 0.5 meV. Percentage absorption was 100% minus the percentage excreted, without correction for recycling, when such small doses of $^{47}$Ca are used. In all studies less than 1% of the $^{47}$Ca dose was excreted in the specimen collected on day 6. During period 2 (February 15, 1969–March 15, 1969), experimental subjects were exposed to the test lights ('Vita-Lite' in ultraviolet-reflective fixtures, 500 foot candles) for 8 h/day. Control subjects were similarly exposed to cool-white fluorescent bulbs in standard luminaries (30–50 foot candles) representing their normal light environment. For the remainder of their waking hours, all subjects lived under the same ambient lighting as during period 1. At the end of this period, $^{47}$Ca absorption was again measured. In period 3 (March 21, 1969–April 25, 1969) all subjects were exposed to the same conditions of artificial light as during period 1. Statistical analyses were made using the paired $t$ test for intragroup changes and the unpaired $t$ test for intergroup changes.

At the end of period 1 $^{47}$Ca absorption was similar in control and experimental groups (39±9% and 41±10% mean ±s.d.). The ranges (23–54%) were those reported for other normal subjects[12–14].

During period 2, mean, $^{47}$Ca absorption in the control group decreased to 30±8%, but increased to 47±9% in the men exposed to the test lights ($P<0.01$). Serum and urinary calcium were unchanged. The decline in the control group probably reflects a continuing decrease in body vitamin D activity resulting from dietary changes and the unavailability of ultraviolet radiation. This suggests that period 1 (49 days) was not long enough to attain a new steady state in calcium absorption. The increased $^{47}$Ca absorption in men exposed to test lights suggests that the small amount of ultraviolet radiation from these lights was sufficient to stimulate significant vitamin D synthesis in the skin, not only preventing the continued depletion of the vitamin, but enhancing the body supply. The changes in vitamin D or 25-OH vitamin D needed to account for such results are unclear, for the dose–response curve relating vitamin D or 25-OH vitamin D to intestinal $^{47}$Ca absorption is undefined in man.

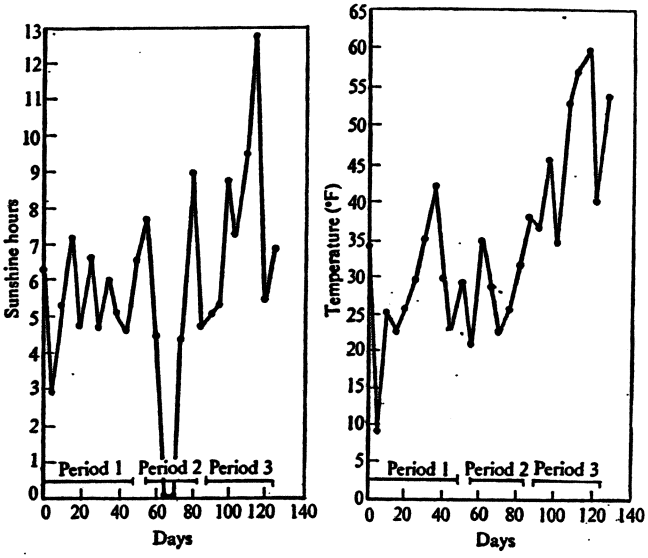


Fig. 2 Sunshine hours and average temperature plotted every fifth day throughout the period December 20, 1968, to April 25, 1969.

The effect of the test lights may be unrelated to their ultraviolet emission or to increased vitamin D synthesis. 'Vita-Lite' resembles natural Sun and sky radiation and differs from standard cool-white fluorescent lighting in not only the ultraviolet but also the visible emission. The intensity of the visible radiation was also much greater for the test group (500 foot candles) than for the control subjects (30–50 foot candles) during period 2. The neuroendocrine apparatus is sensitive to such differences in lighting[15–20], and so it is possible, if unlikely, that variations in the visible portion of the spectrum mediated the differences in calcium absorption.

By the end of period 3, $^{47}$Ca absorption in the control group had also increased significantly (51±7%; $P<0.01$ intra-group) while in the experimental subjects it was virtually unchanged (49±7%). Absorption of $^{47}$Ca in both the control ($P<0.01$) and experimental ($P<0.05$) groups was significantly higher at the end of period 3 than at the end of period 1. The changes in the control group during period 3 cannot be attributed to the artificial lighting, which was the same as in period 1. Further studies will be necessary to define the uncontrolled variable during period 3, but one of the leading possibilities is external illumination. During period 3, the weather in Boston was considerably sunnier than during the first two periods (Fig. 2). Moreover, the average amount of

ultraviolet energy per hour of noonday sunlight reaching Boston during period 3 was three to five times as great as during periods 1 and 2 (refs. 10, 21). At least one man went outside; others sat looking southward through windows which were sometimes open. It seems likely that these men received significant doses of natural ultraviolet light.

These results suggest that relatively small amounts of ultraviolet light can stimulate calcium absorption among elderly men who have no exposure to sunlight and eat a diet containing few foods fortified with vitamin D. Further studies are necessary to confirm these findings and to define the population groups to which they may apply. It is possible that the ability to absorb dietary calcium might be enhanced in large sections of the population by exposure to small amounts of ultraviolet light provided by the general lighting system. The amounts involved seem to be roughly equivalent to those that would impinge on a resident of Washington DC who took a daily 15 min walk out of doors at lunchtime in midsummer.

The changes which occurred between period 1 and period 3 could be construed as evidence that there is seasonal variation in calcium absorption and perhaps vitamin D synthesis, at least among individuals who are exposed to little sunlight.

We thank Mr Sergei Marketan, Duro-Test Corp., North Bergen, New Jersey, who designed and installed the lighting systems, and Dr James Coyle and the staff of the Chelsea Soldiers' Home for their help and cooperation.

R. M. NEER
T. R. A. DAVIS
A. WALCOTT
S. KOSKI
P. SCHEPIS
I. TAYLOR
L. THORINGTON
R. J. WURTMAN

*Massachusetts General Hospital,*
*Boston,*
*Massachusetts* 02114

Received March 17; revised September 14, 1970.

1 Fieser, L., and Fieser, M., *The Steroids*, 90 (Reinhold, New York, 1959).
2 Ponchon, G., Kennan, A. L., and DeLuca, H. F., *J. Clin. Invest.*, 48, 2032 (1969).
3 DeLuca, H. F., *Vitamins and Hormones*, 25, 315 (1967).
4 Hess, A. F., and Anderson, W. T., *J. Amer. Med. Assoc.*, 89, 1222 (1927).
5 Gorter, E., *J. Pediat.*, 4, 1 (1934).
6 Bunker, J. W. M., and Harris, R. S., *New Engl. J. Med.*, 216, 165 (1937).
7 Knudson, A., and Benford, F., *J. Biol. Chem.*, 124, 287 (1938).
8 *Committee on Nutrition, American Academy of Pediatrics, Pediatrics*, 31, 512 (1963).
9 Loomis, W. F. *Science*, 157, 501 (1967).
10 Hollaender, A. (ed.), *Radiation Biology*, 2, 113 (McGraw-Hill, New York, 1955).
11 A.M.A. Council on Foods and Nutrition, *J. Amer. Med. Assoc.*, 185, 588 (1963).
12 Jaworski, L. F., Brown, E. M., Fedoruk, S., and Seitz, H., *New Engl. J. Med.*, 269, 1103 (1963).
13 DeGrazia, J., and Rich, C., *Metabolism*, 13, 650 (1964).
14 Avioli, L. V., McDonald, J. E., Singer, R. A., and Henneman, P. H., *J. Clin. Invest.*, 44, 128 (1965).
15 Fiske, V. M., *Endocrinology*, 29, 187 (1941).
16 Zacharias, L., and Wurtman, R. J., *Obstet. Gynecol.*, 33, 603 (1969).
17 Wurtman, R. J., in *Neuroendocrinology*, 2 (edit. by Martini, L., and Ganong, W. F.), 20 (Academic Press, New York, 1967).
18 Wurtman, R. J., Axelrod, J., and Fischer, J. E., *Science*, 143, 1328 (1964).
19 Moore, R. Y., Heller, R. A., Wurtman, R. J., and Axelrod, J., *Science*, 155, 220 (1967).
20 Wurtman, R. J., Axelrod, J., and Kelly, D. E., *The Pineal* (Academic Press, New York, 1968).
21 Luckiesch, M., *Applications of Germicidal, Erythemal, and Infrared Energy*, 50 (D. Van Nostrand Co., New York, 1946).

# Visna Virus: a Slow Virus with an RNA Dependent DNA Polymerase

VISNA virus is the cause of a progressive neurological disease of sheep which results in paralysis and death[1,2]. After inoculation, the virus multiplies within the host, but illness may not be manifest for months or years[3]. Visna virus shares some physical, chemical and biological properties with oncogenic RNA viruses[4]. The similarities include virion and core size, site of maturation, growth properties, nucleic acid composition[5], and sensitivity to physical and chemical agents. The inhibition of visna virus replication by low concentrations of actinomycin D (ref. 5) indicates a requirement for DNA synthesis for the growth of this agent. An RNA dependent DNA polymerase has been discovered in many oncogenic RNA viruses[6,7]. This communication demonstrates the presence of similar enzyme activity in visna, a virus without known oncogenic potential.

Visna virus and sheep anti-visna serum were provided by Dr Halldor Thormar of the Institute for Basic Research, Staten Island. The virus was plaque purified and propagated in a sheep testes cell strain. Neutralization by the sheep antiserum established its identity. Supernatant culture fluids from visna-infected cell monolayers were clarified by low speed centrifugation and concentrated with 10% polyethylene glycol (Union Carbide, PEG-6000) in the presence of 0.5 M NaCl. This material was then either pelleted through 10% (w/v) sucrose or purified by banding in a preformed continuous gradient of 20–70% (w/v) sucrose for 18 h at 257,000*g* in an SW-65 rotor. The final titre of visna virus used in polymerase assays averaged $10^8$ $TCID_{50}$/ml. The virus used in these experiments was free from contaminating mycoplasma and known murine oncogenic RNA viruses.

**Table 1** Requirements for RNA Dependent DNA Polymerase in Visna Virus

| | Δpmol $^3$H-TTP incorporated |
|---|---|
| Complete | 0.50 |
| +RNase A (1.0 mg/ml.) | 0.20 |
| −dATP | 0.02 |
| −dATP, dGTP, dCTP | 0.02 |
| +Poly rA.rU | 4.10 |
| +Poly rA.rU−dATP, dCTP, dGTP | 4.05 |
| −Virus | 0.02 |
| −Virus+poly rA.rU | 0.02 |
| −DTT | 0.02 |
| $-Mg^{2+}$ | 0.02 |
| −'Triton' | <0.01 |

Each reaction mixture was incubated for 90 min at 37° C and contained in the complete system in 0.05 ml.: 0.04 M Tris HCl (*p*H 7.8); 0.06 M potassium chloride; 0.01 M magnesium acetate; $5\times10^{-4}$ M dATP, dCTP, dGTP; $2\times10^{-5}$ M $^3$H-methylthymidine triphosphate (6,000 c.p.m./pmol); $8\times10^{-3}$ M dithiothreitol; 0.22 $A_{260}$ poly rA.rU where indicated; 0.1% 'Triton X-100' (% v/v) and $10^6$ $TCID_{50}$ of virus. RNase A was heated for 15 min at 90° C to inactivate any possible contaminating DNase. The zero time value in the complete system of 0.07 pmol has been subtracted from all values. $^3$H-methylthymidine triphosphate was purchased from Schwarz Bioresearch; poly rA.rU and deoxynucleoside triphosphates from Miles; RNase A from Calbiochem; DNA synthesis was assayed as previously described[6,7].

Table 1 shows the requirements for the visna virus DNA polymerase. A seven-fold increase in the incorporation of $^3$H-TTP into DNA over the zero time value can be seen. Maximal DNA synthesis required all four deoxynucleoside triphosphates, a divalent cation, 'Triton X-100', and a sulphydryl agent. DNA synthesis was inhibited by RNase A treatment of detergent-disrupted virus suggesting that the template was RNA.

These findings are similar to those observed with the RNA dependent DNA polymerase of C-type oncogenic viruses[6,7]. Recently synthetic RNA polymers have been used to stimulate DNA polymerase activity in C-type oncogenic viruses[8]. Table 1 also shows that one of these templates, poly rA.rU, was