# Rabbits fed on β-carotene have higher serum levels of all-*trans* retinoic acid than those receiving no β-carotene

BY Y. FOLMAN[1]*, R. M. RUSSELL[2], G. W. TANG[2] AND G. WOLF[1]†‡

[1] *Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 and* [2] *USDA–Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, USA*

The aim of the present work was to determine the effect of giving increasing doses of supplements of β-carotene on serum retinoic acid levels in rabbits. Four groups of 7-week-old female rabbits were fed for 9 weeks on a pelleted diet containing 1·72 mg vitamin A as retinyl acetate/kg and including control gelatin beadlets devoid of β-carotene or 1, 2 or 4 mg β-carotene/kg body-weight per d. Serum was collected at 3, 6 and 9 weeks after the beginning of the experiment and the concentration of all-*trans* retinoic acid was determined by a gradient reverse-phase high-performance liquid chromatography system following a double-phase extraction. The average concentration of retinoic acid in serum of the combined control and 1 mg β-carotene/kg groups was 3·80, 3·06 and 2·40 nM at 3, 6 and 9 weeks respectively. The concentrations of retinoic acid in serum of the combined 2 and 4 mg β-carotene/kg groups were 4·80 nM ($P < 0.05$), 3·76 nM (not significant) and 4·90 nM ($P < 0.005$) at 3, 6 and 9 weeks respectively. A SAS (SAS Institute Inc., 1985) general linear model repeated-measures analysis of variance revealed that the effects of treatment ($P < 0.01$), time ($P < 0.05$) and treatment × time interaction ($P < 0.05$) were statistically significant. It is concluded that giving β-carotene is associated with higher concentrations of all-*trans* retinoic acid in the serum of rabbits than in those receiving no β-carotene.

β-Carotene: Retinoic acid: Rabbit.

It has been shown in various studies that retinol and retinaldehyde are converted into all-*trans* retinoic acid (retinoic acid) in animal tissue (Mahadevan *et al.* 1962; Dunagin *et al.* 1964; Crain *et al.* 1967; Emerick *et al.* 1967; Kleiner-Bossaler & De Luca, 1971; Frolik *et al.* 1981). Retinoic acid was detected and measured in animal tissue and fluid following the administration of retinol, retinaldehyde and retinoic acid itself. In humans, retinoic acid was measured under physiological conditions (De Leenheer *et al.* 1982; Lambert & De Leenheer, 1985; Napoli *et al.* 1985). Retinoic acid is, therefore, regarded as a natural metabolite of retinol and retinaldehyde (Frolik, 1984; Cullum & Zile, 1985). Retinoic acid fulfils the function of vitamin A in the processes of growth and epithelial cell maintenance, but not in those of reproduction or vision (Dowling & Wald, 1960; Thompson *et al.* 1969; Ott & Lachance, 1979). On the other hand, retinoic acid is more active than retinol in many in vitro bioassay systems (Roberts & Sporn, 1984).

It has been reported that β-carotene could be converted into retinoic acid by animal tissues in vitro (Crain *et al.* 1967; Napoli & Race, 1988). However, it has not yet been shown that giving β-carotene affects the concentrations of retinoic acid in body fluids or

---

* On sabbatical leave from: Institute of Animal Science, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel.

† Present address: Department of Nutritional Sciences, University of California at Berkeley, Berkeley, CA 94720, USA.

‡ For reprints.

tissues. The present communication provides findings which indicate that giving $\beta$-carotene orally is associated with higher serum levels of retinoic acid in rabbits.

## MATERIALS AND METHODS
### Animals and diet

New Zealand white female rabbits were purchased from Hazelton Research Co. (Denver, PA) at the age of 6 weeks. Before their delivery they had free access to commercial pellets containing lucerne (*Medicago sativa*). On their arrival the rabbits were housed in individual cages and fed on the basic pelleted diet, especially prepared by Bioserv Inc. (Frenchtown, NJ), containing (g/kg): crushed barley grain 200, crushed oat grain 200, sucrose 186, cellulose 140, soya-bean extract 150, AIN-76 mineral mix 70, vitamin mix 20, maize oil 30, methionine 3, choline chloride 0·1. The vitamin mix supplied (mg/kg diet): 1·72 vitamin A as retinyl acetate, 12·5 $\mu$g vitamin D, 20 thiamin, 20 riboflavin, 20 pyridoxine hydrochloride, 10 vitamin K as menadione, 10 Ca D-pantothenate, 10 folic acid, 0·5 biotin, 200 niacin, 40 vitamin E, 50 $\mu$g vitamin $B_{12}$, made up in sucrose to give 20 g. The diet was kept in a 4° cold room and given to the rabbits *ad lib.*

At 1 week after their arrival the rabbits were allocated to groups (seven to eight animals per group) by the method of block randomization: quartets of rabbits identical or similar in their body-weight and feed intake were formed; within the block, the animals were allocated to groups at random. The group mean body-weights were 1·63, 1·66, 1·59 and 1·62 kg. Daily feed intakes during the week before beginning the experiment were 117, 113, 117 and 118 g/d for the four groups. One animal died as the result of an accident. The animals were weighed once weekly; the amount of feed given and the amount refused were also determined.

The protocol of the experiment was designed so that the animals of the four groups would consume 0, 1, 2 and 4 mg $\beta$-carotene/kg body-weight per d in addition to a daily intake of approximately 100 $\mu$g vitamin A/kg body-weight per d. The $\beta$-carotene was given as gelatin beadlets containing 100 mg $\beta$-carotene/g (Rovimix; Hoffman-La Roche, Inc., Nutley, NJ). The zero-carotene diet contained identical gelatin beadlets without $\beta$-carotene, from the same source. Three diets were prepared by mixing into the previously mentioned basic diet: (A) 1·5 g gelatin beadlets without $\beta$-carotene (zero carotene/kg), (B) 0·15 g Rovimix beadlets plus 1·35 g zero-carotene beadlets (15 mg $\beta$-carotene/kg), (C) 1·5 g Rovimix beadlets (150 mg $\beta$-carotene/kg). Rabbits in the zero-carotene group were fed on zero-carotene beadlets only (diet A). Rabbits of the three other groups received each week individually prepared mixtures of the diets A, B and C in such proportions that the planned consumption of $\beta$-carotene was achieved. Each week during feed dispensation the amount of $\beta$-carotene offered was adjusted to the amount consumed during the previous week. Thus, one group of rabbits consumed zero $\beta$-carotene, the second group consumed 1 mg $\beta$-carotene/kg body-weight per d, the third group consumed 2 mg $\beta$-carotene/kg body-weight per d and the fourth group, 4 mg $\beta$-carotene per kg body-weight/d. During the 9 weeks of the experiment, daily feed intake for all the animals ($n$ 29) was 116·3 (SD 15·0) g. Body-weight at the beginning of the experiment was 1·63 (SD 0·14) kg and at the end 3·32 (SD 0·37) kg. The rabbits were bled from the ears 3, 6 and 9 weeks after the beginning of the experiment. The blood was centrifuged, the serum separated and immediately frozen at $-70°$. The serum was used for the determination of retinoids.

### Retinoic acid determination

Ethanol (4·0 ml) was added to serum (2·0 ml), and the tube was vortexed and then centrifuged. After discarding the protein precipitate, sodium hydroxide (2 M, 1·0 ml) was

added to the supernatant fraction and kept for 10 min in an ice-bath and then extracted with n-hexane (5·0 ml). The two phases were separated by centrifugation. To the aqueous phase, hexane (5·0 ml) was added and the mixture centrifuged. The hexane layer was discarded. The aqueous layer was acidified with 2·0 ml 2 M-hydrochloric acid for 10 min in an ice-bath. Then hexane (5·0 ml) was added, and the tube vortexed and centrifuged. The hexane phase was separated from the aqueous phase, and the aqueous layer re-extracted. The two hexane layers were combined and evaporated under nitrogen. The residue was redissolved in 200 $\mu$l methanol and a 100 $\mu$l portion was injected on to the high-performance liquid chromatography (HPLC) column.

An external standard was used to determine the retinoic acid level in serum. Portions of standard of all-*trans* retinoic acid (E $_{1\,cm}^{1\%}$ 1480) solution in methanol (15 ng/ml) were injected on to the HPLC system and peak areas were determined. The peak area of retinoic acid in the serum was compared with that of standard retinoic acid, and the amount present was then calculated. All extractions and handling of retinoic acid were carried out under red light. On the addition of all-*trans* retinoic acid (10 ng) to serum (2·0 ml, human) followed by extraction and analysis, the recovery was 78 (SD 2)% (n 9). Results were not corrected for recovery of standard. Precision of the assay was 93%. Total retinyl esters in serum were measured as one peak (Bankson *et al.* 1986). A gradient reverse-phase HPLC system consisting of a 150 × 4·6 nm Ultrasphere ODS (5 $\mu$m) column and a precolumn packed with hypersil-ODS (10 $\mu$m) were used. The gradient was selected to separate all-*trans* retinoic acid from other polar retinoids. The mobile phase consisted of two solvents, 100% methanol, and methanol–water (65:35, v/v) containing 5 g ammonium acetate/l. The second solvent was used for 1 min followed by a 15 min linear gradient to 100% methanol. Following a 9 min hold at 100% methanol, there was a 5 min gradient back to methanol–water (65:35, v/v). Solvents were sonicated daily and a stream of $N_2$ was allowed to bubble through the solvent during the HPLC run. For detection, Perkin-Elmer LC-95 UV/visible spectrophotometer detector (18 $\mu$l cell) set at 350 nm was used at the maximum sensitivity (0·001 absorbence units, full scale).

### Statistical analysis

Statistical analysis of the data was carried out by the SAS (SAS Institute Inc., 1985) general linear model (GLM) analysis of variance. A SAS GLM procedure for repeated-measures analysis of variance (SAS Institute Inc., 1985) was also carried out. The *post hoc* test after the ANOVA was that suggested by Williams (1972) for the comparison of several dose levels with a zero-dose control.

### RESULTS

The vitamin A status of the animals was unremarkable: mean serum retinol did not differ between treatments or week of experiment, and ranged between 2·45 and 3·57 $\mu$M. Serum retinyl esters, also, did not differ between treatments or weeks of experiment, thus indicating no signs of hypervitaminosis A, even in the high $\beta$-carotene rabbits: control levels were 0·53 (SD 0·21), 0·49 (SD 0·38) and 0·66 (SD 0·75) $\mu$M retinyl esters at 3, 6 and 9 weeks respectively, calculated as retinyl palmitate. In the treatment groups, mean values ranged from 0·61 (SE 0·32) to 1·66 (SE 4·38) $\mu$M; however, due to the high standard error, the differences were not statistically significant. Liver retinol, as expected, showed no differences at 9 weeks (Table 1), whereas liver retinyl esters in the high $\beta$-carotene group only, showed considerable storage of retinyl esters. All serum samples were tested for $\beta$-carotene; it was undetectable (limits of detection, $2 \times 10^{-5}$ $\mu$M).

The average serum concentrations of retinoic acid are shown in Table 2. At 3 weeks after

Table 1. *Liver concentrations of retinol and retinyl esters in rabbits fed on diets providing varying levels of β-carotene† for 9 weeks*

(Values are means with their standard errors)

| Level of β-carotene given (mg/kg body-wt per d) | Liver (nmol/g) | | Liver retinyl esters (as retinyl palmitate) (nmol/g) | |
| --- | --- | --- | --- | --- |
| | Mean | SEM | Mean | SEM |
| 0 | 115 | 17 | 154 | 25 |
| 1 | 115 | 9 | 164 | 30 |
| 2 | 125 | 13 | 162 | 21 |
| 4 | 171 | 17 | 371 | 42** |

Mean value was significantly different from that for zero-carotene control (Williams' test): ** $P < 0.01$.
† For details of diets and dietary regimen, see p. 196.

Table 2. *Serum concentration of all-trans retinoic acid in rabbits fed on diets providing varying levels of β-carotene†*

(Values are means with their standard errors for no. of rabbits shown in parentheses)

| Period on β-carotene (weeks)... Level of β-carotene given (mg/kg body-wt per d) | Serum retinoic acid (nM) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 3 | | 6 | | 9 | |
| | Mean | SEM | Mean | SEM | Mean | SEM |
| 0 | 3·60 | 0·47 (5) | 3·16 | 0·50 (6) | 2·60 | 0·50 (4) |
| 1 | 4·16 | 0·30 (4) | 2·96 | 0·40 (7) | 2·33 | 0·70 (4) |
| 2 | 4·90 | 0·30 (3) | 4·10 | 0·60 (6) | 4·43 | 0·70 (4) |
| 4 | 4·83 | 0·50 (2) | 3·46 | 0·53 (7) | 5·27* | 0·87 (5) |

Mean value was significantly different from zero-carotene control (Williams' test): * $P < 0.05$.
† For details of diets and dietary regimen, see p. 196.

the beginning of β-carotene feeding the levels of retinoic acid were higher by 35% in the groups fed on 2 and 4 mg β-carotene/kg body-weight per d than in the zero β-carotene group, although this difference was not significant. Between 3 and 6 weeks after the beginning of the experiment the concentrations of retinoic acid decreased in all treatment groups; however, this difference was not statistically significant. Between 6 and 9 weeks, levels of retinoic acid continued to decline in the 0 and 1 mg β-carotene/kg body-weight per d groups. However, they increased in the groups fed on 2 or 4 mg β-carotene/kg body-weight per d. At 9 weeks after giving the experimental diets the difference between the zero control and the 4 mg β-carotene/kg body-weight per d group reached statistical significance.

The serum levels of retinoic acid of the group fed on 1 mg β-carotene/kg body-weight per d were similar to those of the zero β-carotene group throughout the experimental period. Likewise, the serum levels of retinoic acid of the group fed on 2 mg β-carotene/kg body-weight per d were similar to those of the group fed on 4 mg β-carotene/kg body-weight per d. The results of these two pairs of groups are shown in Table 3. The justification for combining groups in Table 3 is that the zero β-carotene and the 1 mg β-carotene/kg body-weight per d groups were very similar both at 6 and 9 weeks, and were not statistically

Table 3. *A comparison of serum levels of all-*trans *retinoic acid between rabbits fed on diets providing low and high levels of $\beta$-carotene*†

(Values are means with their standard errors for no. of rabbits shown in parentheses)

| Period on $\beta$-carotene (weeks)... | Serum retinoic acid (nM) | | | | | |
| Level of $\beta$-carotene given | 3 (14) | | 6 (26) | | 9 (17) | |
| (mg/kg body-wt per d) | Mean | SEM | Mean | SEM | Mean | SEM |
| --- | --- | --- | --- | --- | --- | --- |
| 0 and 1 mg | 3·8 | 0·30 | 3·06 | 0·30 | 2·40 | 0·40 |
| 2 and 4 mg | 4·8* | 0·23 | 3·76 | 0·40 | 4·90** | 0·56 |

Mean values were significantly different from those at 0 and 1 mg $\beta$-carotene/kg body-wt per d: * $P < 0.05$, ** $P < 0.005$.

different; the same is true for the 2 and 4 mg $\beta$-carotene/kg body-weight per d groups. Since the values from the two pooled groups are remarkably similar to each other, they could thus be pooled. It can be seen that in this comparison the serum levels of retinoic acid differed significantly 3 weeks after the beginning of the experiment, and at 9 weeks the statistical significance of the difference increased to $P < 0.005$. An analysis by the SAS GLM procedure for repeated-measures analysis of variance revealed a highly significant treatment effect ($P < 0.01$), a significant time effect ($P < 0.05$) and a time × treatment interaction ($P < 0.05$). The results of the analysis indicate that the decrease with time in the serum concentration of retinoic acid (Table 2) is significant, but that it occurs only in some of the treatments. Hence the significant interaction indicated a significant decrease in the animals of the groups fed on 0 and 1 mg $\beta$-carotene/kg body-weight per d and no decrease in the groups fed on 2 and 4 mg $\beta$-carotene/kg body-weight per d.

The values presented in Tables 2 and 3 indicate that rabbits fed on the two higher levels of $\beta$-carotene showed significantly higher levels of retinoic acid in serum in comparison with the groups given no $\beta$-carotene or a low level of $\beta$-carotene. Thus, the serum levels of retinoic acid depend on the dose of $\beta$-carotene, i.e. on the level of $\beta$-carotene in the diet. It should be noted that the amount of retinoic acid measured included a derivative of retinoic acid, retinoyl $\beta$-glucuronide, which is hydrolysed to retinoic acid by the base used in the extraction procedure (Barua & Olson, 1986).

## DISCUSSION

The serum and liver retinol and the serum retinyl ester concentration of the rabbits did not statistically differ between the various treatment groups. Serum retinol levels were higher than those found in rats. Serum retinyl ester levels were also elevated: no doubt because the animals were not fasted and were, therefore, in a continuous postprandial state. Only the liver retinyl ester concentration of the 4 mg $\beta$-carotene/kg body-weight per d group was significantly higher than that of the three other groups. The retinyl ester concentration of the 2 mg $\beta$-carotene/kg body-weight per d group was almost identical to that of the 0 and 1 mg $\beta$-carotene/kg body-weight per d groups (Table 1), but the serum concentration of retinoic acid in the 2 mg $\beta$-carotene/kg body-weight per d group was similar to that of the 4 mg $\beta$-carotene/kg body-weight per d group. It is thus plausible to assume that the higher retinoic acid levels in the 2 and 4 mg $\beta$-carotene/kg body-weight per d groups were not the result of an increased retinol or retinyl ester conversion. Furthermore, Napoli & Race (1988) show results, at least in rats in vitro, which exclude the formation of retinoic acid

from the retinol produced by cleavage of $\beta$-carotene. It is, therefore, suggested that the higher levels of retinoic acid concentration found in the present study are the result of $\beta$-carotene conversion.

Since $\beta$-carotene is cleaved to retinal on its way to retinol, the intermediate retinal may be converted to retinoic acid. A conversion of $\beta$-carotene to retinoic acid in the gut was first described by Crain et al. (1967). More recently, Napoli & Race (1988) reported the quantitatively significant conversion of $\beta$-carotene to retinoic acid by cytosolic enzymes of a number of tissues. Retinoic acid increases in human plasma after an oral dose of retinoic acid (Jurkowitz, 1962) and, together with the retinoid glucuronides (Barua & Olson, 1986), is an endogenous steady-state metabolite (De Ruyter et al. 1979). Thus, it is not unlikely that the increased retinoic acid in serum is derived from the dietary $\beta$-carotene.

The gradual decrease during the experiment in the serum levels of retinoic acid in the control group may have been caused by the high $\beta$-carotene liver stores at the time the animals were purchased. The animals consumed approximately 1·5 mg $\beta$-carotene/kg body-weight per d before arrival at our laboratory. Giving a diet supplying 0 or 1 mg $\beta$-carotene/kg body-weight per d may have caused enough of a decrease in $\beta$-carotene stores to result in a decline in serum levels of retinoic acid. Shapiro et al. (1984) found that rats accumulated considerable amounts of $\beta$-carotene in some specific tissues (e.g. heart) when fed on a similar level of $\beta$-carotene (0·02 g/kg diet) over 2 weeks. Rabbits are 'white fat' animals, similar to rats with respect to $\beta$-carotene storage. In any case the observed decline in retinoic acid was not statistically significant.

The biological significance of the fact that dietary $\beta$-carotene causes increased steady-state blood levels of retinoic acid is at present unclear. It was previously suggested that retinoic acid may have a different biological function from that of retinol (Zile & Cullum, 1983; Wolf, 1984). In this context the conversion of $\beta$-carotene into retinoic acid may have biological importance. If, as suggested previously, retinol and retinyl ester may not be the easily available precursors of retinoic acid under normal physiological conditions, then $\beta$-carotene may be an important source of retinoic acid supplementation. The very rapid metabolic clearance of retinoic acid from body fluids and tissues (55–85 % of a physiological dose excreted in bile in 24 h; for review, see Frolik, 1984) may increase the significance of this conversion. The effect of $\beta$-carotene on fertility is of special interest, since the pituitary and the female and male reproductive organs were found to contain high concentrations of cellular retinoic acid-binding protein (Ong et al. 1982). Thus, the possibility exists that the reported effects of $\beta$-carotene on fertility in women and cows (Kemmann et al. 1983; Folman et al. 1983, 1987) may be mediated by retinoic acid produced from $\beta$-carotene.

The anti-carcinogenic action of $\beta$-carotene, reported both in animal and epidemiologic studies (for review, see Greenberg et al. 1985), could possibly be caused by previous conversion to retinoic acid. This possibility should be considered when pondering the apparent paradox that increased dietary vitamin A (in the form of $\beta$-carotene) appears to lower the risk of cancer (Greenberg et al. 1985), even though serum blood levels of retinol remain unchanged. The increase in dietary $\beta$-carotene may by-pass retinol in the gut and produce increased retinoic acid, which could be the actual anti-carcinogenic factor.

REFERENCES

Bankson, D. D., Russell, R. M. & Sadowski, J. A. (1986). Determination of retinyl esters and retinol in serum and plasma by normal phase chromatography: methods and applications. Clinical Chemistry 32, 35–40.

Barua, A. B. & Olson, J. A. (1986). Retinyl β-glucuronide, an endogenous compound of human blood. *American Journal of Clinical Nutrition* **43**, 481–485.

Crain, F. D., Lotspeich, F. J. & Krause, R. F. (1967). Biosynthesis of retinoic acid by intestinal enzymes of the rat. *Journal of Lipid Research* **8**, 249–254.

Cullum, M. E. & Zile, M. H. (1985). Metabolism of all-*trans*-retinoic acid and all-*trans* retinyl acetate. *Journal of Biological Chemistry* **260**, 10590–10596.

De Leenheer, A. P., Lambert, W. E. & Claeys, I. (1982). All-*trans* retinoic acid: measurement of reference values in human serum by high performance liquid chromatography. *Journal of Lipid Research* **23**, 1362–1367.

De Ruyter, M. G., Lambert, W. E. & De Leenheer, A. P. (1979). Retinoic acid: an endogenous compound of human blood. *Analytical Biochemistry* **98**, 402–409.

Dowling, J. E. & Wald, G. (1960). The biological function of vitamin A acid. *Proceedings of the National Academy of Sciences, USA* **46**, 587–608.

Dunagin, P. E., Zachman, R. D. & Olson, J. A. (1964). Identification of free and conjugated retinoic acid as a product of retinal (vitamin A aldehyde) metabolism in the rat *in vivo*. *Biochimica et Biophysica Acta* **90**, 432–434.

Emerick, R. J., Zile, M. & De Luca, H. F. (1967). Formation of retinoic acid from retinol in the rat. *Biochemical Journal* **102**, 606–611.

Folman, Y., Ascarelli, I., Kraus, D. & Barash, H. (1987). Adverse effect of β-carotene on fertility of dairy cows. *Journal of Dairy Science* **70**, 357–366.

Folman, Y., Rosenberg, M., Ascarelli, I., Kaim, M. & Herz, Z. (1983). The effect of dietary and climatic factors on fertility and on plasma progesterone and oestradiol-17β-levels in dairy cows. *Journal of Steroid Biochemistry* **19**, 863–868.

Frolik, C. A. (1984). Metabolism of retinoids. In *The Retinoids*, vol. 2, pp. 177–208 [M. B. Sporn, A. B. Roberts and D. S. Goodman, editors]. Orlando: Academic Press.

Frolik, C. A., Dart, L. L. & Sporn, M. B. (1981). Metabolism of all-*trans* retinyl acetate to retinoic acid in hamster tracheal organ culture. *Biochemica et Biophysica Acta* **663**, 329–335.

Greenberg, E. R., Baron, J. A. & Beck, J. R. (1985). Carotenoids and cancer prevention. In *Retinoids: New Trends in Research and Therapy*, pp. 360–370 [J. Saurat, editor]. Basel: S. Karger.

Jurkowitz, L. (1962). Determination of vitamin A in human plasma after oral administration. *Archives of Biochemistry and Biophysics* **98**, 337–341.

Kemmann, E., Pasquale, S. A. & Skaf, R. (1983). Amenorrhea associated with carotenemia. *Journal of the American Medical Association* **249**, 926–929.

Kleiner-Bossaler, A. & De Luca, H. F. (1971). Formation of retinoic acid from retinol in the kidney. *Archives of Biochemistry and Biophysics* **142**, 371–377.

Lambert, W. E. & De Leenheer, A. P. (1985). Demonstration of retinoic acid isomers in human urine under physiological conditions. *Experientia* **41**, 359–360.

Mahadevan, S., Murthy, S. K. & Ganguly, J. (1962). Enzymic oxidation of vitamin A aldehyde to vitamin A acid by rat liver. *Biochemical Journal* **85**, 326–331.

Napoli, J. L., Pramanik, B. C., Williams, J. B., Dawson, M. I. & Hobbs, P. D. (1985). Quantification of retinoic acid by gas–liquid chromatography–mass spectrometry: total versus all-*trans*-retinoic acid in human plasma. *Journal of Lipid Research* **26**, 387–392.

Napoli, J. L. & Race, K. R. (1988). Biogenesis of retinoic acid from β-carotene. *Journal of Biological Chemistry* **263**, 17372–17377.

Ong, D. E., Crow, J. A. & Chytil, F. (1982). Radioimmunochemical determination of cellular retinol and cellular retinoic acid-binding proteins in cytosol of rat tissue. *Journal of Biological Chemistry* **257**, 13385–13389.

Ott, D. B. & Lachance, P. A. (1979). Retinoic acid—a review. *American Journal of Clinical Nutrition* **32**, 2522–2531.

Roberts, A. B. & Sporn, M. B. (1984). Cellular biology and biochemistry of the retinoids. In *The Retinoids*, vol. 2, pp. 209–286 [M. B. Sporn, A. B. Roberts and D. S. Goodman, editors]. Orlando: Academic Press.

SAS Institute Inc. (1985). *SAS User's Guide: Statistics*, version 5, pp. 478–483. Cary, North Carolina: SAS Institute Press.

Shapiro, S. S., Mott, D. J. & Machlin, L. J. (1984). Kinetic characteristics of β-carotene uptake and depletion in rat tissue. *Journal of Nutrition* **114**, 1924–1933.

Thompson, J. N., Howell, J. McC., Pitt, G. A. J. & McLaughlin, C. I. (1969). The biological activity of retinoic acid in the domestic fowl and the effects of vitamin A deficiency on the chick embryo. *British Journal of Nutrition* **23**, 471–490.

Williams, D. A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519–531.

Wolf, G. (1984). Multiple functions of vitamin A. *Physiological Reviews* **64**, 873–937.

Zile, M. H. & Cullum, M. E. (1983). The function of vitamin A: current concepts. *Proceedings of the Society for Experimental Biology and Medicine* **172**, 139–152.