

of the Headmaster of the school, and we are most grateful to him. He and others of his staff very kindly provided accommodation for the members of the Medical Research Council Group who lived at the school during the investigation. The Domestic Science Mistress and the Caterer were at all times willing to give information about the school food, and to help in many other ways. Finally, we wish to thank most warmly the boys who took part in the investigation, although it caused considerable disruption of their routine.

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Metabolism of Carotene and Vitamin A Given by Mouth or Vein in Oily Solution or Aqueous Dispersion to Calves, Rabbits and Rats

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It is now generally accepted that in many species of the higher animals carotene taken by mouth is converted to vitamin A in the intestinal wall (see, for example, review by Kon & Thompson, 1951). There is both less agreement and less evidence on the value as vitamin A precursor of carotene given parenterally (cf. Church, MacVicar, Bieri, Baker & Pope, 1954). The observations of Tomarelli, Charney & Bernhart (1946) about the relation between the utilization of carotene introduced by injection and its state of dispersion offer an explanation of the divergence of findings. The careful work of Bieri & Sandman (1951) and Bieri & Pollard (1954) with rats, of Hentges, Grummer & Sorensen (1952) with pigs, of Church *et al.* (1954) with sheep, and of Bieri (1955) with chicks and rabbits, shows beyond reasonable doubt that these animals under suitable conditions convert into vitamin A carotene introduced directly into the body. In all these experiments carotene was dispersed in water-miscible surface-active agents. With the same technique Eaton, Matterson, Decker, Helmboldt & Jungherr (1951) found conversion in the calf, but Church *et al.* (1954) failed to do so.

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opened and as much blood as possible was taken in an oxalated syringe directly from the heart. The rats were then killed and the required organs were taken for analysis.

Rabbits

All the rabbits used were on normal diets rich in carotene and received additional vitamins A and D. They were between the ages of 3 months and 3 years when used. Some were Belgian Blacks, the remainder mainly Chinchilla Giganta \times Old English.

The dispersions of carotene or vitamin A were injected into a vein in the left ear and samples of blood were obtained simultaneously or later from the corresponding vein of the other ear. Dosing by mouth was done by placing the dose at the back of the tongue with a short length of polythene tubing and a syringe. The final treatment of the rabbits was as described for rats.

Calves

All calves were male, three of them Ayrshires and the remainder Shorthorns; they were collected at birth before they had suckled. Some calves received 3 pt. of twice separated colostrum and then the synthetic diet of Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell (1949) from which vitamin A was omitted. Other calves were obtained at 3 weeks of age; up to that time they had received an initial allowance of 6 pt. whole colostrum during the first 24 h and then whole milk. They were then maintained on the synthetic diet of Aschaffenburg *et al.* (1949) till 8–12 weeks old, when their plasma values for vitamin A were in the same range (2–10 i.u./100 ml.) as those of the calves put directly on to the diet low in vitamin A. Finally, further calves were used within 3 days of birth, and during that period received the synthetic diet only.

Injections were made into the left jugular vein, and blood samples were taken subsequently from the right jugular vein. For oral administration the dose was mixed with 400 ml. warm separated milk in a Waring Blendor and added to 3 lb. of the normal milk feed. After the final blood sample had been taken, calves were killed by a humane killer or intravenous injection of Nembutal (Abbott Laboratories Ltd), and the required organs were then taken for analysis.

Measurement of vitamin A and carotene in different tissues

All estimations were carried out as described by Thompson *et al.* (1949), except that to obtain more uniform weakening of the alumina used for chromatography it was suspended in 8% ethanol in *n*-hexane before the column was made.

RESULTS

Experiments with rats

Intravenous injection

Carotene. Some difficulty was experienced in ensuring that the whole of the dose was introduced into the tail vein, but the level of carotene in the blood was used as a measure of the efficiency of the injection. The results in Table 1 (Exps. 1 and 2)

Table 1. *Appearance of carotene and vitamin A in the blood and liver of male rats partly deficient in vitamin A, after the intravenous injection of carotene or vitamin A as dispersion in Tween* or colloidal dispersions or oily solutions*

| Exp. no. | Rats | | Dose | | | Site of injection | Time between injection and killing (h) | Blood plasma | | | | Total liver content | | |
|----------|----------|-----------------|----------------------|--------------|-------------------|-------------------|--|--------------|------------------------|----------------------|------------|---------------------|----------------|--------------|
| | No. used | Mean weight (g) | Vehicle | | Substance | | | Vitamin A | | Carotene | | Vitamin A | | |
| | | | Nature | Amount (ml.) | | | | Amount (µg) | Alcohol (i.u./100 ml.) | Ester (i.u./100 ml.) | µg/100 ml. | Total† (µg) | Alcohol (i.u.) | Ester (i.u.) |
| 1 | 2 | 257 | Tween dispersion | 0.2 | — | Tail vein | 4 | 32 | 13 | 0 | 0 | 2.5 | 1.5 | 0 |
| | 4 | 276 | Tween dispersion | 0.2 | Carotene | Tail vein | 4 | 90 | 14 | 90 | 8.4 | 4.5 | 7.3 | 28 |
| | 3 | 263 | Arachis oil | 0.2 | Carotene | Tail vein | 4 | 39 | 15 | 0 | 0 | 2.1 | 2.8 | 0 |
| 2 | 2 | 272‡ | Tween dispersion | 0.4 | — | Tail vein | 2 | 14 | 17 | 0 | 0 | 7.0 | 9.0 | 0 |
| | 4 | — | Tween dispersion | 0.4 | Carotene | Tail vein | 2 | 85 | 25 | 500 | 47 | 15 | 4.5 | 50 |
| | 2§ | — | Tween dispersion | 0.4 | Carotene | Tail vein | 2 | 37 | 23 | 600 | 55 | 12 | 2.2 | 0 |
| | 1§ | — | Tween dispersion | 0.4 | Vitamin A acetate | Portal vein | 2 | 51 | 40 | 1300 | 120 | 14 | 2.9 | 76 |
| 3 | 1 | — | Tween dispersion | 0.4 | — | Tail vein | 2 | 130 | 50 | 0 | 0 | 48 | 240 | 0 |
| | 1 | — | Tween dispersion | 0.4 | — | Portal vein | 1 | 120 | 48 | 0 | 0 | 220 | 58 | 0 |
| | 1 | — | Tween dispersion | 0.4 | — | Vena cava | 1½ | 480 | 130 | 0 | 0 | 220 | 77 | 0 |
| | 2 | 223 | — | — | — | — | — | 15 | 8.0 | 0 | 0 | 3.0 | 1.2 | 0 |
| | 1 | 260 | Tween dispersion | 0.4 | Carotene | Vena cava | 1½ | 35 | 13 | 4800 | 420 | 2.4 | 0.9 | 30 |
| | 1 | 274 | — | — | — | Vena cava | ½ | 29 | 9.0 | 2600 | 240 | 10 | 2.2 | 100 |
| | 1 | 296 | — | — | — | Vena cava | 2 | 60 | 7.6 | 1200 | 130 | 12 | 2.4 | 120 |
| | 1 | 288 | — | — | — | Vena cava | 2 | 76 | 13 | 620 | 61 | 7.0 | 5.6 | 160 |
| | 1 | 262 | Colloidal dispersion | 0.4 | Carotene | Vena cava | 4 | 15 | 7.0 | 1000 | 90 | 3.2 | 1.0 | 210 |
| | 1 | 284 | — | — | — | Vena cava | ½ | 13 | 6.0 | 0 | 0 | 4.0 | 1.7 | 240 |
| | 1 | 320 | — | — | — | Vena cava | 2 | 14 | 5.0 | 0 | 0 | 3.0 | 1.0 | 200 |
| | 1 | 280 | — | — | — | Vena cava | 4 | 13 | 6.0 | 0 | 0 | 2.6 | 1.5 | 240 |
| 4 | 2 | 327 | — | — | — | — | — | 18 | 5.0 | 0 | 0 | 1.9 | 0.6 | 0 |
| | 2 | 318 | Tween dispersion | 0.4 | Carotene | Vena cava | 2 | 140 | 5.0 | 1200 | 130 | 13 | 2.8 | 72 |
| | 4 | 317 | — | — | — | Vena cava | 24 | 54 | 10 | Trace | Trace | 25 | 35 | 110 |

* Tween means a 20% (v/v) solution of Tween 40 in water.

† The figures show the total amount in the blood in µg, calculated on the assumption that a rat contains 6.7 ml. blood/100 g (Cartland & Koch, 1928), of which plasma constitutes 50%.

‡ Mean weight for all rats used in Exp. 2 (range 242–309 g).

§ Rats kept throughout under ether anaesthesia.

|| Female rats.

showing an increase in vitamin A in the blood after injection of carotene in Tween are in agreement with those of Bieri & Pollard (1954). The conversion occurred whether the dispersion was injected into the tail vein or into the portal vein, and with the former whether anaesthesia was maintained throughout or was for a short time only. On the other hand, with oily solutions no conversion was observed. The increase was mainly in the alcohol form, increases in the ester values being relatively small and probably not significant. This result is in contrast with that after administration by mouth, where the initial increase was largely in the ester form (Thompson *et al.* 1949). The increase in liver vitamin A was mainly as vitamin A alcohol after 2 h, but by 4 h the ester predominated; some carotene also appeared in the liver. There were only traces of vitamin A alcohol and ester in the small intestine of control and injected animals.

In further experiments shown in Table 1 (Exps. 3 and 4) the effect was studied of injecting a Tween or a colloidal dispersion of carotene into the vena cava. The results show that, 5 min after injection of the Tween dispersion, most of the carotene was still in the blood, but that it decreased rapidly there during the next 4 h and after 24 h only a trace could be found. Vitamin A appeared at first in the blood and in the liver in the alcohol form, but subsequently the ester form in the liver increased; after 4 h the content of it nearly equalled that of the alcohol form and surpassed it after 24 h.

By contrast with the marked effects with depleted rats, experiments not quoted here in detail showed that injection of carotene in Tween into stock-colony animals with normal concentrations of vitamin A in the blood, increased both alcohol and ester by only some 20%.

When colloidal carotene was injected into the vena cava it disappeared very rapidly from the circulation, so that 5 min after the injection only one-quarter to one-fifth of the dose was recovered; no vitamin A was formed in these conditions. Carotene injected as the colloidal dispersion appeared more rapidly in the liver and reached a higher concentration in it than if injected in Tween dispersion. No vitamin A was found in the lungs after either treatment, but some 2.5 μg carotene appeared there after injection of the colloidal dispersion, and 7.5 μg after injection of the Tween dispersion (means for four rats).

Vitamin A. An experiment with vitamin A acetate dispersed in Tween showed an initially very rapid disappearance from the blood of the injected vitamin. Most of the vitamin A remaining in the blood after 5 min was in the alcohol form (Table 1, Exp. 2). In the liver, vitamin A appeared at first as the alcohol which was gradually replaced by the ester.

Administration by mouth

Carotene. Table 2 shows the results of a feeding experiment where the same amount of carotene in Tween or in oil, mixed with the diet, was given by stomach tube to rats 2 h before killing. With Tween, much higher blood values for vitamin A ester and alcohol and for carotene were achieved than with the oily solutions, liver storage too was greater, but there was no difference in the amount of vitamin A found

Table 2. Appearance of vitamin A and carotene in various organs of female rats partly deficient in vitamin A after a stomach-tube meal of 4 mg carotene in 400 mg arachis oil mixed with 1 g vitamin A-deficient diet and 4 ml. water, or of 4 mg carotene in 4 ml. Tween* dispersion mixed with 1 g diet. Both doses contained 20 mg α -tocopherol. All rats were killed 2 h after dosing

| No. of rats | Mean weight (g) | Intestine, total content | | | | | | | | | | | |
|-------------|------------------------|--------------------------|-----------------------------|----------------|--------------|---------------------|----------------|--------------|---------------------|----------------|--------------|---------------------|-----|
| | | Blood plasma | | | | | Wall | | | | | | |
| | | Vitamin A | | Carotene | Vitamin A | | Vitamin A | | Vitamin A | | Vitamin A | | |
| Dose | Alcohol (i.u./100 ml.) | Ester (i.u./100 ml.) | Carotene (μ g/100 ml.) | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) | |
| 2 | 229 | 14 | 4.0 | 0 | 3.5 | 3.0 | 0 | 1.5 | 1.2 | 0 | 3.5 | 3.0 | 2.5 |
| 5 | 210 | 86 | 52 | 11 | 8.0 | 46 | 38 | 2.4 | 1.2 | 78.0 | 17 | 34 | 8.0 |
| 5 | 205 | 150 | 190 | 66 | 10 | 47 | 52 | 3.6 | 2.6 | 210 | 28 | 60 | 17 |

* Tween means a 20% (v/v) solution of Tween 40 in water.

Table 3. Effect of repeated blood sampling, and of injection of 2 ml. of a 20% (v/v) aqueous solution of Tween into an ear vein of rabbits, on the concentration of vitamin A alcohol in their blood

| Time (h) | Vitamin A alcohol in the blood (i.u./100 ml.) | | | | | | | | | |
|----------------|---|----------|------|------------------------------------|----------|--|------|------------------------------------|--|--|
| | After blood sampling only | | | | | After injection of Tween and subsequent blood sampling | | | | |
| | Rabbit A | Rabbit B | Mean | Difference from initial mean value | Rabbit C | Rabbit D | Mean | Difference from initial mean value | | |
| 0 | 160 | 210 | 180 | — | 120 | 170 | 140 | — | | |
| $\frac{1}{2}$ | — | — | — | — | 150 | 140 | 140 | 0 | | |
| $\frac{1}{4}$ | — | — | — | — | 130 | 140 | 140 | 0 | | |
| $\frac{1}{2}$ | — | 250 | — | — | 120 | — | — | — | | |
| $\frac{3}{4}$ | — | — | — | — | — | 140 | — | — | | |
| 1 | 160 | 240 | 200 | +20 | 120 | 130 | 120 | -20 | | |
| $1\frac{1}{2}$ | 120 | — | — | — | — | — | — | — | | |
| 2 | 170 | 180 | 180 | 0 | 120 | 130 | 120 | -20 | | |
| 6 | 110 | 160 | 140 | -40 | 96 | 140 | 120 | -20 | | |
| 24 | 200 | 210 | 200 | +20 | 110 | 150 | 130 | -10 | | |

in the intestinal wall. With carotene in oil so little carotene appeared in the blood that it could be measured only when the blood from several rats was pooled (cf. Thompson *et al.* 1949).

Experiments with rabbits

Similar experiments were carried out with rabbits. The ease with which repeated blood samples can be obtained from the ear veins allowed the changes in the blood values for carotene and vitamin A to be followed at time intervals as short as $1\frac{1}{2}$ min.

Effect of repeated blood sampling and injection of Tween on the concentration of vitamin A in the blood

In order to establish what effect repeated sampling or the use of Tween as a dispersing agent had on the concentration of vitamin A alcohol or ester in the blood, experiments were done in which repeated blood samples were taken without other treatment or after injection of the 20% solution of Tween alone (Table 3). It will be seen that considerable fluctuations occurred in the concentration of vitamin A alcohol on repeated blood sampling alone. The variations were no greater when 2 ml. Tween were injected into the ear vein, but the trend was towards a drop in the values. Subsequent experiments with four rabbits, each injected on two separate occasions, confirmed the decrease in vitamin A alcohol after Tween alone; the ester values increased slightly or remained unchanged, the mean effect being a rise to 14 i.u./100 ml. from 10.

Intravenous injection of carotene in Tween

Carotenoids do not normally circulate in the blood of the rabbit, though its normal diet is rich in them. The effects of injecting 1 mg carotene in Tween are shown in Fig. 1*a* and of 2 mg in Fig. 1*b*. Rough calculation, based on the assumption that the blood content of the rabbit is 6.2% of the body-weight (Dukes, 1947) and that plasma constitutes 60% of the blood volume, shows that after 5 min most of the dose was in the circulating blood. One hour after dosing the concentration in the blood had fallen appreciably, and this decline continued at a regular rate. Table 4 shows in greater detail the rapidity of the disappearance of carotene from blood after injection and shows that after 20 h most of the carotene had disappeared from it. Table 5 shows that of the carotene injected into rabbits only small amounts appeared in the heart, lungs and kidneys, and that even those disappeared rapidly and completely within the next 3 h. The quantities appearing in the liver were about ten times as large as those in these other organs and much more persistent; in proportion to the dose they were less than in the rat (Table 1). Carotene was not detected in the remainder of the carcass, suggesting that the injected carotene was actually destroyed and not merely removed from the blood and stored in other tissues. The injection of carotene caused an increase in blood vitamin A alcohol, barely measurable after 5 min but amounting to 50–70 i.u./100 ml. plasma* after 1–3 h, the concentration declining to even less than the initial value (range 65–175 i.u./100 ml.) after between 6 and 20 h (Fig. 2).

* As the injection of Tween alone tended to lower vitamin A alcohol in the blood it is possible that the effect of carotene was greater than these values would suggest.

Values for vitamin A ester obtained in these experiments are not given in Fig. 2, as they were unreliable owing to temporary difficulties with impurities in the light petroleum used as solvent.

Later experiments with six rabbits showed a rise also in the ester, the mean increase being to 24 i.u./100 ml. from 8.7, some of it being accounted for no doubt by the increase observed with Tween alone.

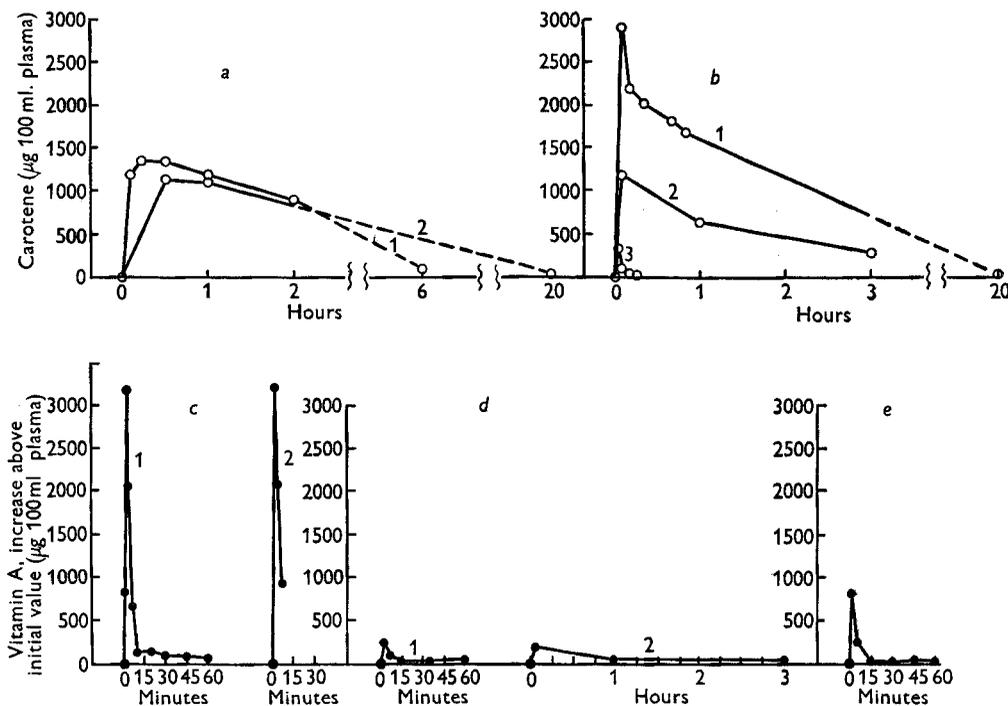


Fig. 1. Effects on blood concentration of carotene and of vitamin A of intravenous injection into rabbits of different preparations of carotene or of vitamin A.

(a) Injection of 1 ml. Tween* dispersion containing 1 mg carotene/ml. Each curve for a single rabbit.

(b) Curves 1 and 2, as a but injection of 2 ml.; curve 3, injection of 2 ml. aqueous colloidal dispersion containing 2 mg carotene. Curves 1 and 3 for single rabbits; in curve 2 each point represents a different rabbit.

(c) Injection of 2 ml. Tween dispersion containing 20 mg vitamin A acetate. Each curve for a single rabbit.

(d) As (c) but dispersion containing 2 mg vitamin A acetate. Curve 1 for a single rabbit; in curve 2 each point represents a different rabbit.

(e) Injection of 2 ml. aqueous colloidal dispersion containing 2 mg vitamin A acetate. Curve for a single rabbit.

Values for vitamin A are given in µg to facilitate direct comparison with those for carotene. Values in i.u. for rabbits 1 and 2 in (c) can be obtained from Table 4.

* Tween means a 20% (v/v) solution of Tween 40 in water.

Intravenous injection of vitamin A in Tween

The rapid disappearance of injected carotene prompted investigation into the fate of vitamin A injected in the same way. Fig. 1d and Table 5 show that, within 5 min of the injection of 2 mg vitamin A acetate, only a small proportion of the dose was

found in the blood or other organs. In order to study more easily this rapid rate of disappearance ten times the dose of vitamin A acetate in the same quantity of Tween was injected and the changes in the blood values were followed at short time intervals. These findings are shown in Fig. 1c and in detail in Table 4. It can be roughly calculated (see p. 250) that even within 0.5–2 min only one-sixth of the injected dose could be recovered in the blood, and after 5–7 min only from one-twentieth to one-thirtieth of the dose remained there. The rapid disappearance of vitamin A from the blood might be explained through rapid uptake by other organs. The results shown

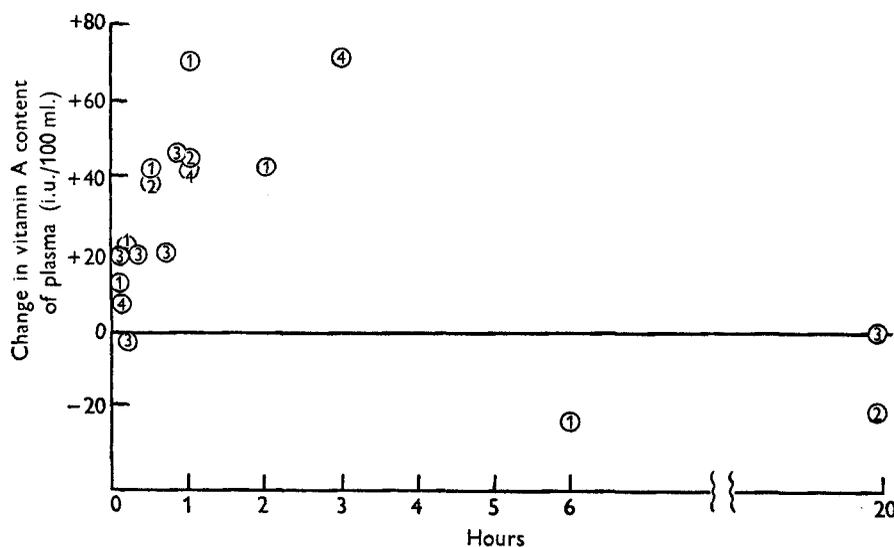


Fig. 2. Scatter diagram of change in vitamin A content of blood plasma of rabbits after intravenous injection of carotene in Tween* dispersion.

① Injection of 1 ml. Tween dispersion containing 1 mg carotene/ml. Same rabbit as in Fig. 1a curve 1.

② As ①, but same rabbit as in Fig. 1a, curve 2.

③ As ①, but injection of 2 ml. Same rabbit as in Fig. 1b, curve 1.

④ As ③, but same rabbit as in Fig. 1b, curve 2.

—, base-line. Mean initial value 110 i.u./100 ml. (range 65–180 i.u./100 ml.).

* Tween means a 20% (v/v) solution of Tween 40 in water.

in Table 5 demonstrate that after 5 min, apart from the liver, the largest quantity of vitamin A was in the lungs, but that after 1 h it had decreased there to one-tenth of the value after 5 min. Analyses of the carcass and other organs showed that the injected vitamin A must in fact have been destroyed. It is striking that this rapid destruction occurred only with injected vitamin A, for Table 3 shows no such dramatic effect of Tween itself on the vitamin A normally present in the circulation. Although the acetate was used for injection, the vitamin was recovered in all tissues mainly in the alcohol form. The de-esterification of the acetate was extremely rapid, for in one instance, shown in Table 4, a sample of blood was taken during injection, which lasted about 30 sec, and already the blood value was greatly increased by equal quantities of vitamin A alcohol and ester.

Intravenous injection of colloidal carotene and vitamin A

Carotene in colloidal form disappeared from the blood after injection much more rapidly than that dispersed in Tween. Already 1.5 min after the injection only a small fraction of the dose was found in the circulation, and after 1 h no demonstrable carotene was present (Fig. 1*b*, curve 3). The results for blood showed no evidence of formation of vitamin A; although in the liver vitamin A was apparently increased

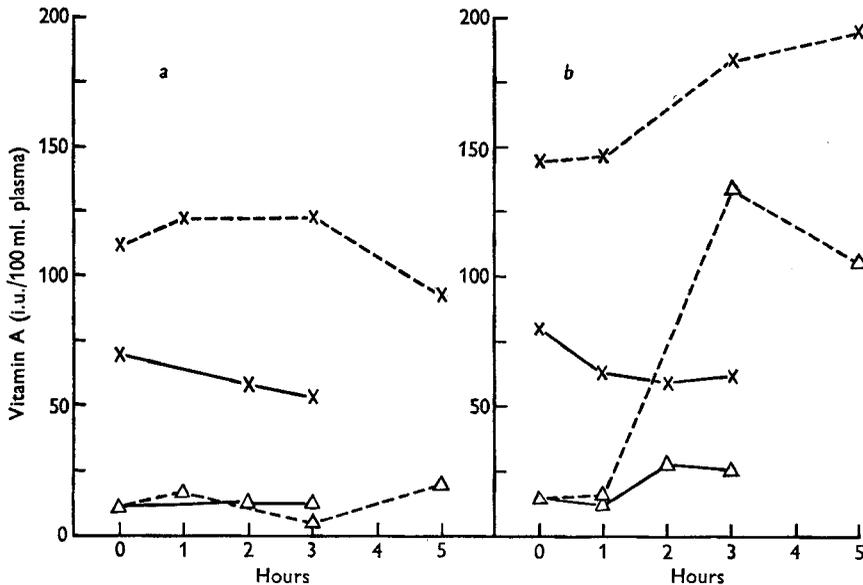


Fig. 3. Effect on blood concentration of vitamin A alcohol and ester of different preparations of carotene or vitamin A given to rabbits by mouth.

(a) Administration of 3 mg vitamin A acetate in 5 g arachis oil or of 10 mg carotene in 1 g arachis oil.

(b) As a, but in 10 ml. Tween* dispersion.

x, vitamin A alcohol; Δ, vitamin A ester; broken curves, administration of vitamin A acetate (one rabbit); solid curves, administration of carotene (mean for three rabbits).

* Tween means a 20% (v/v) solution of Tween 40 in water.

(Table 5), this increase may well be within the range of variation for these rabbits which were not deficient. Of the organs studied (Table 5), the liver was the main site of deposition of the carotene injected in the two ways, the next highest amount being found in the lungs. In both organs more of the colloidal carotene than of that in Tween was deposited.

With injection of vitamin A, the blood value 1.5 min later was distinctly greater for the colloidal form (Fig. 1*e*), but thereafter with both forms vitamin A rapidly disappeared from the blood, and after 30 min basal values were observed with both colloidal and Tween dispersions.

Oral administration of carotene and vitamin A

Fig. 3*a* shows that rabbits utilized oily solutions of carotene or vitamin A extremely poorly; in fact 10,000 i.u. of vitamin A in oil caused only a small increase in blood

vitamin A ester or alcohol. Hence it is not surprising that 10 mg carotene in oil had no demonstrable effect during the 3 h of the experiment. However, carotene in Tween increased the ester form of vitamin A in the blood (Fig. 3*b*), and vitamin A in Tween dispersion (Fig. 3*b*) increased both the ester and the alcohol. The better utilization of carotene in Tween was evident also from analysis of the wall of the small intestine. In three rabbits given carotene in oil the intestine contained 15 i.u. vitamin A and 2.4 μ g carotene 3 h after dosing, whereas for three other animals similarly given the Tween dispersion, the values were 91 i.u. and 13 μ g, respectively.

During these experiments we noticed wide fluctuations in the initial concentration of the ester in the blood of untreated rabbits. Inquiry showed that high values were connected with recent consumption of kale, which contributed most of the carotene in the ration. When kale was intentionally withheld or offered, the mean findings for groups of two rabbits were as follows:

| Exp. no. | Diet | Days on diet | Vitamin A ester (i.u./100 ml. plasma) | Vitamin A alcohol (i.u./100 ml. plasma) |
|----------|--------------|--------------|---------------------------------------|---|
| 1 | With kale | — | 16 | 190 |
| | Without kale | 1 | 11 | 160 |
| | | 3 | 5.2 | 160 |
| | | 14 | 5.1 | 180 |
| 2 | Without kale | 4 | 5.4 | 180 |
| | With kale | 1 | 28 | 200 |
| | | 3 | 16 | 200 |
| | | 14 | 23 | 240 |

It is evident that the concentration of the ester was relatively much more influenced than that of the alcohol.

Experiments with calves

Toxic effects of Tween injected intravenously

Rabbits, injected intravenously with Tween in the quantities usual for administration of carotene or vitamin A, frequently towards the end of the injection showed momentary signs of discomfort or distress, but injection of such a solution of carotene or of Tween alone, into the jugular vein of calves on the same weight basis (80 mg of Tween itself/lb. body-weight) were promptly fatal to the first two animals so treated. Two further calves died within 5–15 min though given vitamin A in only half that dose of Tween. The Tween content of the dose of carotene or vitamin A had finally to be reduced to 20 mg/lb. body-weight to be tolerated by the calves without undue reaction. It was not possible, therefore, to give the calves more than one-eighth, on the weight basis, of the dose of carotene given to the rats.

Krantz, Culver, Carr & Jones (1951) found respiratory failure in rats poisoned by injection of Tween. In the present investigation, the calves that died after injection showed signs of asphyxia, frothing freely through the nose and mouth, and at autopsy the lungs had nearly twice the normal weight owing to oedema.

Intravenous injection of carotene in Tween

Each of three calves (nos. 2-4) was injected on three occasions at intervals of 6-10 days. The results varied little from one calf to another, so a composite curve is given (Fig. 4*a*) in which the times of each set of injections were made to coincide and each point was based on all values available for the given time after injection. The figures on the graph represent the number of calves whenever less than three were contributing to the curve.

Calculations similar to those reported for the rat (Table 1) and the rabbit (p. 250), on the basis that in the calf blood constitutes 7.7% of the body-weight (Dukes, 1947) and contains 50% plasma, show that at the earliest sampling, 5-15 min after injection, only about half the dose of carotene was in the circulating blood. Rapid deposition in other sites may well account for much of the remainder. Thus, one of the calves killed by the injection of excessive amounts of a Tween dispersion of carotene (see above) had, of a total of some 40 mg, 4 mg in the liver and 8 mg in the lungs. Fig. 4*a* shows the subsequent rapid decrease of the blood carotene at a rate similar to that in the rat and rabbit (Fig. 5). In contrast with the rat and rabbit, a small secondary rise occurred in the calves from 3 to 24 h after the injection. Church *et al.* (1954) noticed a similar tendency in the calves with which they worked. Thereafter, the carotene value remained for at least 10 days at about 75 $\mu\text{g}/100$ ml. plasma.

Three further calves (nos 5-7) were injected with carotene in Tween and mean values for them are plotted in Fig. 4*b*. The initial rise in blood carotene was similar to that observed in the first three calves, but the secondary rise was barely noticeable and, moreover, the subsequent carotene value was much lower, about 15 $\mu\text{g}/100$ ml. plasma. It is not possible to say whether the discrepancies in the behaviour of the two groups of calves were in any way connected with the partial crystallization of carotene in the second sample of carotene in Tween.

The noteworthy feature of these experiments was the almost complete lack in all six calves of any effect of the injection of carotene on the plasma value for vitamin A, as will be seen from Fig. 4*a, b*, where the vitamin A alcohol of the plasma is plotted. The small but reproducible increase in vitamin A alcohol after each injection may indicate a very limited conversion of carotene, too limited to be of value to the calf. The ester values, not given in the figure, remained consistently at 1-2 i.u./100 ml. plasma. Furthermore, the reserves of vitamin A in the liver, kidneys and lungs of the injected calves were not sensibly different from those of the control (Table 6). By contrast, carotene absent from the organs of the control animal was present in those of the treated; the relatively large quantities in the lungs are noteworthy.

Intravenous injection of carotene in colloidal dispersion

Three calves (nos. 8-10) were injected once. After 5 min, the shortest period studied, and after 3 h, no carotene was detectable in the blood (Fig. 4*c*). Thereafter, carotene began to appear in increasing quantities and reached at slaughter (48 h) a concentration of 16 $\mu\text{g}/100$ ml. plasma. Table 6 shows that in these calves, the amount of carotene

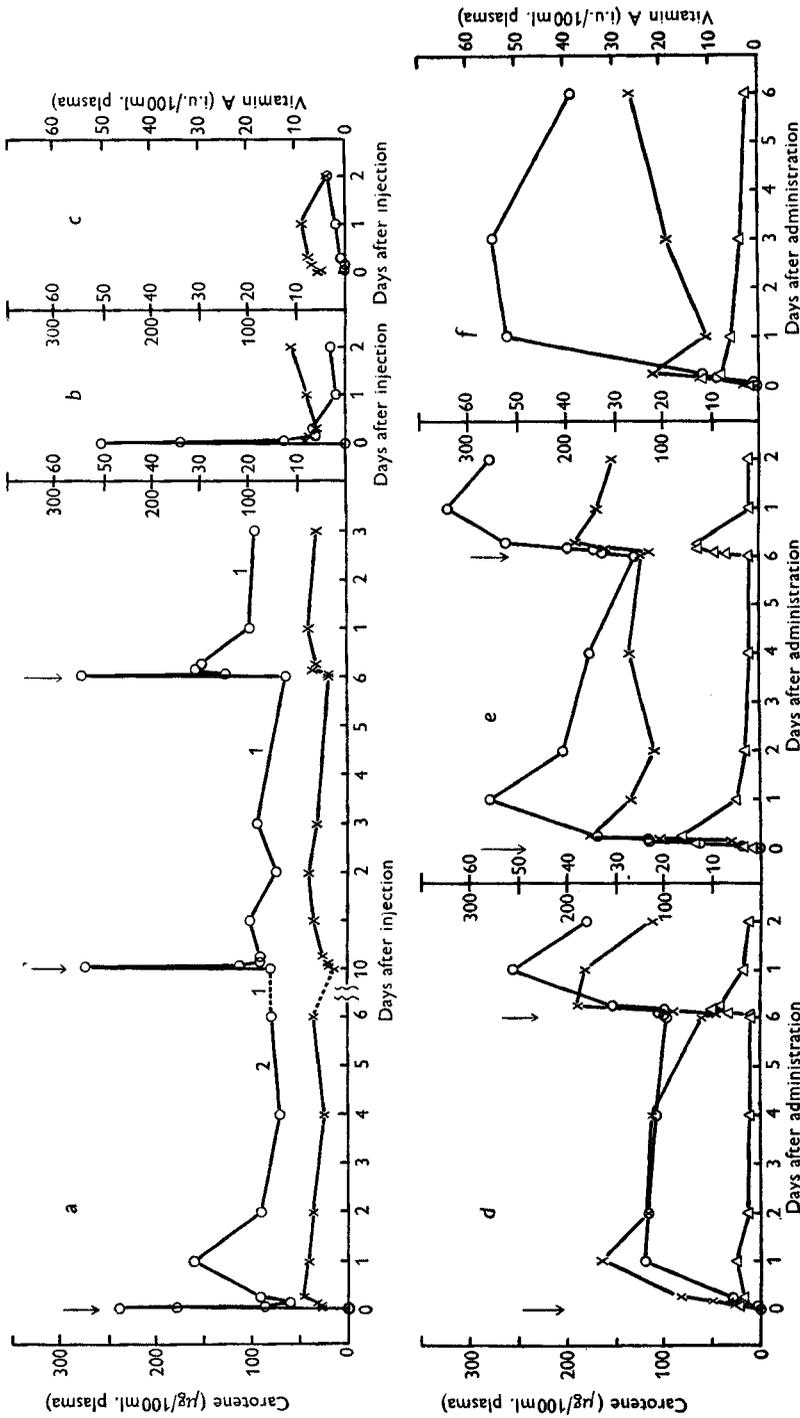


Fig. 4. Effect on blood concentration of carotene and of vitamin A-alcohol and ester of different preparations of carotene injected into calves or given to them by mouth.

- (a) Repeated injection of 10 ml. Tween* dispersion containing 10 mg carotene. Composite curve for three calves (nos. 2-4).
- (b) As a but single injection. Calves nos. 5-7.
- (c) Single injection of 10 ml. aqueous colloidal dispersion containing 10 mg carotene. Composite curve for three calves (nos. 8-10).
- (d) Repeated administration by mouth in the feed of 40 g arachis oil containing 400 mg carotene and mixed with 400 ml. warm separated milk. Composite curves for two calves (nos. 12 and 13).
- (e) Repeated administration by mouth in the feed of 400 ml. Tween dispersion containing 400 mg carotene and mixed with 400 ml. warm separated milk. Composite curves for two calves (nos. 14 and 15).
- (f) Single administration by mouth in the feed of 300 ml. of an arachis oil emulsion containing 400 mg carotene and mixed with 400 ml. warm separated milk. Composite curve for two calves (nos. A and 5).

Arrows indicate repeated injection or administration by mouth, and figures on a the number of calves when less than three.

○—○, carotene; ×—×, vitamin A alcohol; △—△, vitamin A ester.
* Tween means a 20% (v/v) solution of Tween 40 in water.

in the liver was about one-third, and in the lungs about three times, that in the calves injected with the Tween dispersion. With colloidal carotene, as with carotene in Tween, there was no evidence of formation of vitamin A.

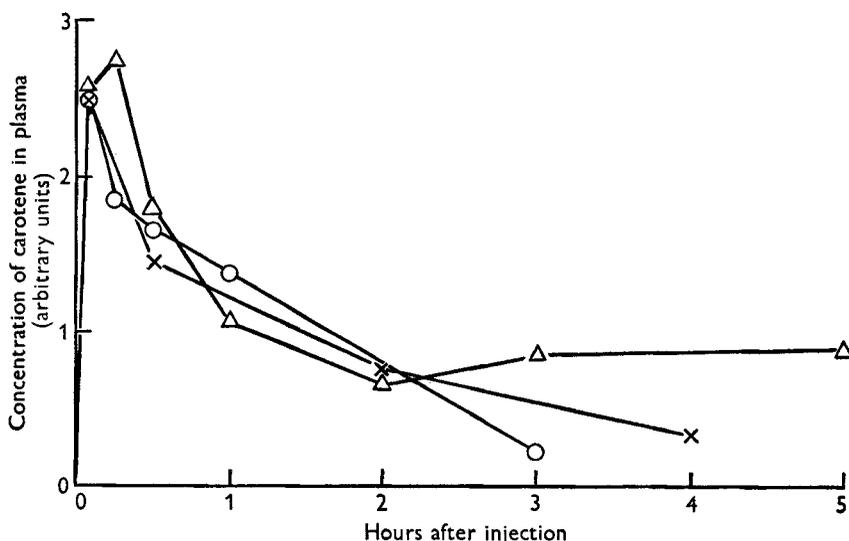


Fig. 5. Changes in carotene concentration in blood plasma in the calf, rat and rabbit after intravenous injection of carotene in Tween*. For purposes of comparison the first readings for the rat and rabbit 5 min after injection were transformed to arbitrary units so as to be numerically identical with the corresponding value for the calf, and all other values for the two first species were then transformed by the use of the same factors. All three curves begin at the origin. Δ — Δ , calf, based on composite values for calves nos. 2-4 (see Fig. 4*a* and Table 6); \times — \times , rat, based on values in Table 1, Exp. 3, for four rats, one rat per point; \circ — \circ , rabbit, based on values for rabbit given in Table 4, Exp. 3.

* Tween means a 20% (v/v) solution of Tween 40 in water.

Intravenous injection of vitamin A in Tween

The rapid disappearance of vitamin A in Tween injected intravenously into rabbits made of interest a similar study with calves. Two calves (nos. 11 and 16) received into the jugular vein an injection of 20 mg vitamin A acetate in Tween. The mean findings are given in Fig. 6*a*. Although the vitamin was injected as the ester, both alcohol and ester increased markedly in the blood at the first sampling 5 min after injection. The ester values fell nearly to the initial values within 1 h. The concentration of the alcohol form decreased between 5 and 15 min after injection and then increased and remained steady for a few hours. This increase was similar to, though earlier than, that found after carotene injection. One of the calves was killed 4 days after injection. The other was kept for a further 12 days, at the end of which the concentration of vitamin A alcohol in its blood was down to the initial value. In the first calf the total vitamin A present in the liver, lungs and kidneys (Table 6) represented storage of only one-tenth of the dose. The two calves that died 5-15 min after injection of 20 mg vitamin A in Tween (see p. 255) had on the average 3700 i.u. vitamin A in the lungs

Table 6. Total content of carotene and vitamin A in various organs of calves after administration of different preparations of carotene or of vitamin A acetate by intravenous injection or by mouth

| Exp. no. | Vehicle | | Dose | | Substance | Amount (mg) | Mode of administration | Calf no. | Time between last injection and killing (days) | Liver | | | Lungs | | | Kidneys | | |
|----------|------------------------------|--------------|-------------------|-------------|-----------|-------------|------------------------|----------|--|----------------|--------------|---------------------|----------------|--------------|---------------------|----------------|--------------|---------------------|
| | Nature | Amount (ml.) | Nature | Amount (mg) | | | | | | Vitamin A | | | Vitamin A | | | Vitamin A | | |
| | | | | | | | | | | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) | Alcohol (i.u.) | Ester (i.u.) | Carotene (μ g) |
| 1 | Tween* dispersion | 10 | — | Carotene | 3 × 10 | By vein | 1 | 0 | 100 | 100 | 0 | 31 | 30 | 0 | 70 | 40 | 0 | |
| | | | | | | | | | 150 | 260 | 750 | 60 | 110 | 560 | 89 | 37 | — | |
| | | | | | | | | | 150 | 380 | 980 | 34 | 10 | 120 | 54 | 6.0 | 21 | |
| | | | | | | | | | 130 | 160 | 1,000 | 32 | 26 | 870 | 80 | 28 | 62 | |
| 2 | Tween dispersion | 10 | Carotene | 1 × 10 | By vein | 5† | 2 | 77 | 260 | 2,600 | 47 | 24 | 540 | 100 | 7.5 | 26 | | |
| | | | | | | | | 60 | 170 | 770 | 29 | 29 | 390 | 45 | 14 | 14 | | |
| | | | | | | | | 81 | 65 | 530 | 27 | 47 | 330 | 29 | 8.3 | 0 | | |
| 3 | Aqueous colloidal dispersion | 10 | Carotene | 1 × 10 | By vein | 8 | 2 | 59 | 39 | 280 | 63 | 31 | 1800 | 91 | 6.3 | 0 | | |
| | | | | | | | | 60 | 19 | 200 | 66 | 26 | 1600 | 52 | 9.0 | 0 | | |
| | | | | | | | | 52 | 18 | 140 | 30 | 15 | 780 | 29 | 9.4 | 0 | | |
| 4 | Tween dispersion | 10 | Vitamin A acetate | 1 × 20 | By vein | 11 | 4 | 740 | 6,200 | 0 | 100 | 65 | 0 | 200 | 45 | 0 | | |
| | | | | | | | | 490 | 7,900 | 880 | 32 | 15 | 75 | 370 | 28 | 86 | | |
| 5 | Oily solution | † | Carotene | 2 × 400 | By mouth | 13 | 2 | 520 | 6,900 | 2,100 | 40 | 16 | 150 | 120 | 15 | 68 | | |
| | | | | | | | | 360 | 6,200 | 19,000 | 41 | 160 | 650 | 150 | 30 | 69 | | |
| 6 | Tween dispersion | † | Carotene | 2 × 400 | By mouth | 15 | 2 | 1000 | 7,500 | 15,000 | 47 | 18 | 180 | 210 | 12 | 43 | | |
| | | | | | | | | 1500 | 160,000 | 0 | 86 | 48 | 0 | 640 | 30 | 0 | | |
| 7 | Oily solution | † | Vitamin A acetate | 1 × 300 | By mouth | 16§ | 6 | 1500 | 160,000 | 0 | 86 | 48 | 0 | 640 | 30 | 0 | | |

* Tween means a 20% (v/v) solution of Tween 40 in water.

† Had received 1 month earlier carotene emulsion by mouth.

‡ See text, p. 246.

§ Had received 16 days earlier the same treatment as calf no. 11.

and 700 i.u. in the kidneys, almost entirely as vitamin A alcohol; in contrast the liver contained 12,000 i.u. mainly as the ester. The small quantity of froth collected contained per 100 ml. 2600 i.u. vitamin A alcohol and 700 i.u. ester.

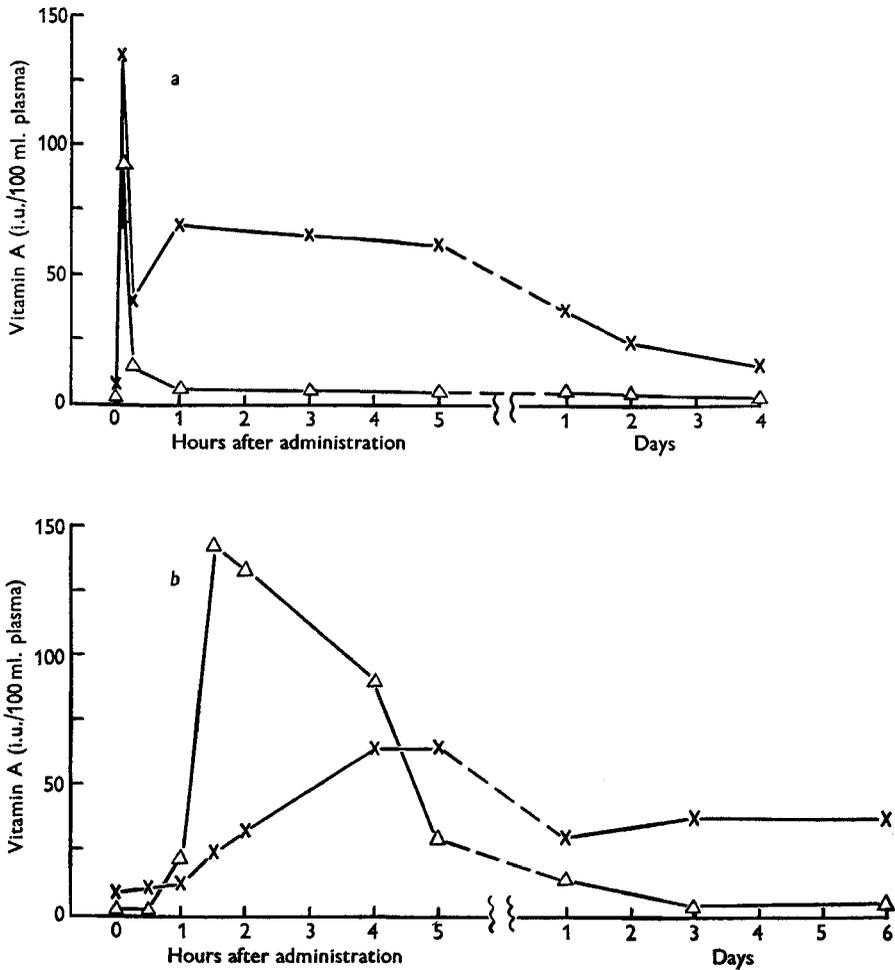


Fig. 6. Effect on blood concentration of vitamin A alcohol and ester of vitamin A injected into calves or given to them by mouth.

(a) Injection into calves nos. 11 and 16 of 10 ml. Tween* dispersion containing 20 mg vitamin A acetate.

(b) Administration by mouth in the feed to calf no. 16† of 50g arachis oil containing 1,000,000 i.u. vitamin A as fish-liver oil concentrate, and mixed with 400 ml. warm separated milk.

x—x, vitamin A alcohol; \triangle — \triangle , vitamin A ester.

* Tween means a 20% (v/v) solution of Tween 40 in water.

† Sixteen days after treatment a.

Administration by mouth of carotene or vitamin A

Tween dispersions, colloidal dispersions, oily solutions or aqueous emulsions of oily solutions of carotene. Table 6 and Fig. 4d-f show the effects of the administration by mouth of carotene in different carriers. The quantity of carotene so given was forty

times that injected (see Table 6), i.e. 400 mg. With carotene in oil and with the Tween dispersion dosing was repeated after 6 days (calves nos. 12-15). It will be seen that ingestion of carotene in Tween resulted in an earlier and greater increase in blood carotene and vitamin A ester than ingestion of carotene in oil. The difference with vitamin A alcohol was less marked. At the second dosing the general picture was the same; of the two forms the ester increased first, 1-2 h after dosing, the increase in the alcohol following 2-4 h after dosing. Earlier experiments with three calves given by mouth the same dose of carotene in oil, and with two similarly given colloidal carotene, and all observed for only 24 h, showed a very similar picture for the behaviour of carotene and of vitamin A ester and alcohol in the blood, and also the same time relationships in the appearance of the two forms.

It is noteworthy that several other calves that received carotene in oil while suffering from scours failed to absorb or convert the carotene, for neither it nor vitamin A appeared in the blood. In fact, the oily solution was usually passed as such in the faeces.

There was no difference between the two treatments (Tween and oil) in the magnitude of the liver stores of vitamin A or in the concentrations in the lungs and kidneys (Table 6). On the other hand, the calves given the Tween dispersion stored some ten times more carotene in the liver. When emulsified oily carotene was given to two calves (nos. A and 5, Fig. 4*f*) the carotene in the blood reached a level as high as after Tween dispersion and maintained it longer, but the increase in both forms of vitamin A was little different from that after carotene in oil. The liver reserves were not measured.

Oily solutions of vitamin A. Fig. 7 presents the results of the administration by mouth of 1 million i.u. vitamin A as 1 oz. mixed fish-liver oils to each of seventeen newborn calves and also values for seven calves not dosed. The calves had been denied natural access to colostrum and received by bucket its non-fatty fraction and 'synthetic' diet (see p. 246).

The most noteworthy feature of the behaviour of these very young animals was the extremely rapid return of the blood values to the initial value, despite the storage of some 100,000 i.u. in the liver. It would seem that a low content of vitamin A in the blood in such young calves is physiological and not an expression of insufficiency of vitamin A.

It is interesting in this connexion that a 3-month-old calf (no. 16, previously injected with a Tween dispersion of vitamin A, see p. 258), given the same dose, maintained for 6 days a blood concentration (Fig. 6*b*) about double that reached after 40 h by the newborn calves (Fig. 7). Its liver stores (Table 6) were of the same order as theirs.

DISCUSSION

The main purpose of this work was to establish whether, and to what extent, carotene introduced directly into the circulation is converted into vitamin A in two species, the rat and the rabbit, in whose blood carotenoids do not normally circulate, and in a third, the calf, that usually has relatively large quantities in the blood. To study the

effect of the state of dispersion carotene was injected in two types of aqueous dispersion, one obtained by means of the surface-active polyoxyethylenesorbitan monopalmitate (Tween 40), the other in water alone. To help in interpreting the findings, especially those in which vitamin A was formed from injected carotene, vitamin A itself was given also in the two dispersions. Finally, oily solutions of carotene also were injected. In a separate category were experiments in which the efficiency of absorption and utilization of carotene and vitamin A given by mouth as oily solutions was compared with that of aqueous dispersions similarly given.

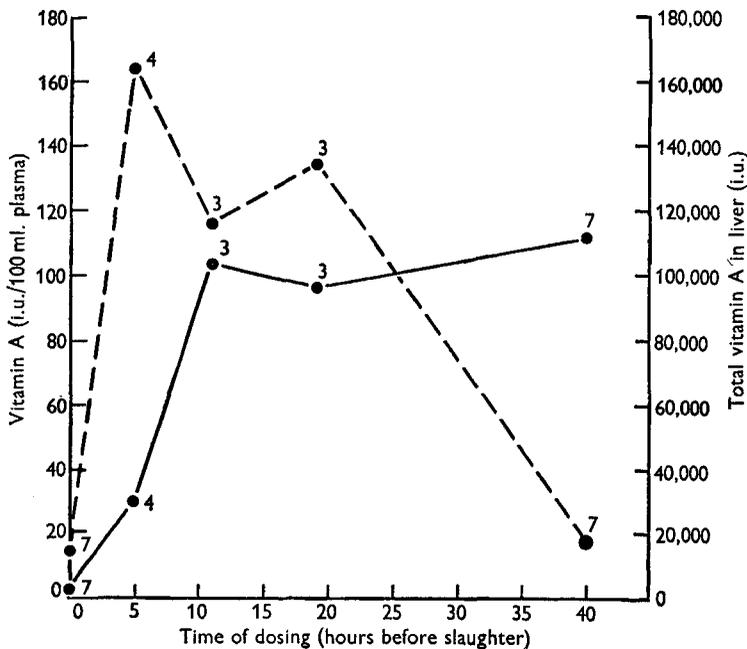


Fig. 7. Mean values for the appearance of vitamin A (alcohol and ester) in the blood plasma and liver of newborn calves (seventeen in all) given in the feed at varying intervals before slaughter a dose of 1,000,000 i.u. vitamin A as 1 oz. mixed fish-liver oil concentrate mixed with 400 ml. warm separated milk. Figures indicate the number of calves contributing to a value; the initial values are those for seven untreated calves. ●—●, liver; ●--●, plasma.

It is known that dispersing agents of the Tween type are toxic when injected in sufficient concentration (see, for example, Krantz *et al.* 1951; Sobel, Rosenberg & Engel, 1952). The quantities of Tween injected into rats and rabbits in the present investigation were larger than those used by Sobel *et al.*, and were of the order of concentrations believed by those authors to produce a slight degree of haemolysis in the rabbit. Apart from the slight and transient reaction observed in some of our rabbits immediately after injection, neither they nor the rats seemed at all affected. Moreover, repeated samples of blood taken from rabbits were never haemolysed to any extent. We were therefore surprised when doses comparable on a weight basis proved rapidly fatal to calves, for which in the end the rate of dosage for the rabbit had to be reduced to one-quarter for Tween to be given with impunity. In the two

calves that died within a few minutes of the injection of the half dose, the quantity of vitamin A present in the lungs was one-third of that in the liver and five times that in the kidneys. The calf that died after injection of carotene in Tween dispersion had, of the total of 40 mg given, 8 mg carotene in the lungs and only 4 mg in the liver. High concentrations of vitamin A were found in the lungs also of rabbits shortly after injection of non-toxic doses of Tween, and it would seem that the lung plays an important part in the metabolism of carotene and vitamin A introduced into the circulation; the destruction of much of the carotene and vitamin A so given to our animals may well have been due to oxidation in the lungs. Shortly after injection most of the carotene was in the blood of rats (Table 1) and rabbits (p. 250), and about half of it was in the blood of calves (p. 256), but we never could account for more than a fraction of the injected vitamin A. It is well known that, of the two, vitamin A is more prone to oxidation, and it did in fact disappear the more rapidly.

Our experiments provide evidence of the formation of vitamin A from injected carotene in rats and in rabbits. The rats were depleted of vitamin A and the evidence rests on the solid basis of a rapid rise in blood vitamin A from low or almost 'blank' values to normal concentrations, and of the appearance of appreciable quantities in the liver.

With the rabbits, which were normally nourished in respect of vitamin A, the evidence is less direct, but in all instances, injection of carotene was followed by an almost immediate and appreciable rise that lasted 3–6 h. Church *et al.* (1954) found a similar rise and return to normal values in wethers injected with carotene. We showed by injecting Tween alone that the appearance of vitamin A in the blood was not due to mobilization of liver stores.

By contrast, all our experiments with calves were negative in that they provided virtually no evidence that vitamin A was formed from injected carotene. Admittedly, the quantity of carotene given to calves at any one injection was, on the weight basis, less than that given to rats or rabbits, but even after three consecutive injections the calves had no more vitamin A in the liver and other organs than those untreated. Our experience with the Dairy Shorthorn confirms therefore that of Church *et al.* with Herefords.

Bieri & Pollard (1954) with the rat, and Hentges *et al.* (1952) with the pig, have shown beyond reasonable doubt that injected carotene, in contrast with that taken by mouth, is transformed into vitamin A away from the intestine. Our evidence is more indirect than that of these authors, but as far as it goes supports their views. Vitamin A appeared in the blood of our deficient rats within 5 min, and in the liver within $\frac{1}{2}$ h, after the injection of carotene. We know from past experience (Thompson *et al.* 1949) that if the carotene had had to pass first from the blood to the intestine to be converted there, vitamin A would not have appeared in the blood for at least $\frac{3}{4}$ h. Moreover, in that event we should have detected vitamin A in the intestinal wall, but there was none in our injected rats.

Further support for conversion of injected carotene elsewhere than in the intestinal wall may be derived from consideration of the form of vitamin A that appears. Table 1 shows that the increase in blood vitamin A that followed the injection of carotene into

rats was very largely of the alcohol form. Comparison of the values in Fig. 2 and on p. 250 of the text shows similar results with the rabbit. It will be recalled that vitamin A arising from intestinal conversion appears in the lymph exclusively as the ester (Thompson *et al.* 1949, 1950) and that the ester form at first predominates in the blood. It might be argued that in the presence of Tween such circulating ester would be broken down to the alcohol, since much of vitamin A acetate injected in Tween was de-esterified in our experiments. However, enough of the ester remained in the circulation to differentiate clearly the state in the blood from that obtaining after injection of carotene. Moreover, our experiments with rabbits (Table 4 and p. 250) show that this change of the ester form to alcohol in the presence of Tween occurred only when the ester was injected in Tween dispersion, but that the ester already circulating in the blood and derived from the food was not affected by the injection of Tween alone or carrying carotene.

Shortly after injection of carotene the alcohol form predominated in the liver of deficient rats, whereas, as shown by us earlier (Thompson *et al.* 1949), both forms appear there in equal amounts after a meal of carotene. This difference in the proportion of the two forms is a further argument to our mind against the possibility that the conversion of injected carotene takes place in the intestine.

We have no positive indication from our work about the organ or site of the extra-intestinal conversion. All we know is that a large part of the carotene that appears initially in the circulation and in the lungs is rapidly destroyed, presumably by oxidative breakdown, with the simultaneous appearance of some vitamin A. We are inclined at present to share the view of Bieri & Pollard (1954) that vitamin A may be one of the products of an oxidative chain and that its formation need not be confined to any particular site, though the lung may well prove to play an important even if not specific part.

Whatever the final explanation, it might well be asked why the calf does not form vitamin A from injected carotene, and some difference in the katabolic pathway of carotene seems a likely answer. Carotene circulates in the blood of cattle, and the fact may be directly linked with their inability to convert injected carotene, a connexion already mentioned by Church *et al.* (1954). It is of interest that, at first, the rate at which injected carotene disappeared from the blood was the same for calves, rats and rabbits (Fig. 5), that in the last two it remained almost unchanged till all carotene had disappeared within 24 h, but that in the calf the concentration of carotene rose slightly about 6 h after injection and thereafter remained fairly constant.

So far only a Tween dispersion of carotene has been considered. The behaviour of carotene dispersed without Tween, and hence of much larger particle size, differed sharply in some respects. Immediately after the injection only a very small portion of the dose appeared in the blood of rats and rabbits and none in that of calves, in which surprisingly, however, it began appearing a few hours after the injection, that is at about the time of the secondary rise which follows the injection of carotene in Tween. It seemed as if in calves some of the carotene was at first stored in some tissue to be released later.

The almost instantaneous disappearance of carotene from the circulation after being

injected in coarser dispersion is only partly explained by its appearance in the liver, where about half the dose was deposited in rats, about a fifth in rabbits but only a fiftieth in calves. For the first two species these proportions were higher than after carotene in Tween, but for the calves, quite strikingly, the reverse was true. Once deposited in the liver, carotene persisted there for some time. Analysis after various time intervals of the carcasses of rats and rabbits showed that the remainder of the dose, whatever the state of dispersion, must in fact have been destroyed very rapidly.

A second and possibly most significant difference in the behaviour of the two kinds of dispersion was the failure by rats and rabbits to form any vitamin A from carotene introduced in the coarser state. It may be that the size of the particles itself prevented the carotene from following the necessary oxidation path; it is possible also that the carotene disappeared from the blood too rapidly to reach a specific site of conversion, if such in fact exists. The fact remains that, once deposited in the liver, the carotene was used not at all, or only to a very limited extent, to form vitamin A, and it is noteworthy in this connexion that carotene was equally persistent and inactive whether injected in Tween or in coarser dispersion, which is perhaps evidence against the liver as a site of conversion.

The finding that vitamin A given by mouth in Tween dispersion was distinctly better utilized than in oily solution is in agreement with numerous reports of earlier workers critically reviewed for example by Sobel (1952). Much less information is available about the behaviour of carotene similarly given, and in fact we are aware of only one paper, that by Burns, Hauge & Quackenbush (1951). Under our conditions carotene too was better absorbed and utilized in Tween dispersion than in oily solution; rats 2 h after dosing had five times as much carotene in the blood as after an equivalent dose in oil, and the vitamin A alcohol and ester in the blood and liver were appreciably higher. With calves, carotene and vitamin A appeared in the blood more rapidly and in greater concentration, and much more carotene was deposited in the liver, though the vitamin A stores were the same.

The total efficiency of utilization of carotene and vitamin A injected in aqueous dispersion is worthy of consideration. Though there is no doubt that doses so given produce initially high concentrations in the blood and other organs, the lability in the animal of such dispersions and especially those of vitamin A leads to great waste of the injected material, so that, with vitamin A at any rate, the efficiency of this mode of administration is perhaps more apparent than real when compared with the slower but less wasteful uptake from oily solution given by mouth. It must naturally be conceded that where there is impairment of digestive function, injection into the circulation may still be preferable.

SUMMARY

1. Vitamin A-deficient rats and calves and normal rabbits were given intravenously aqueous dispersions of carotene and vitamin A in Tween 40 (polyoxyethylenesorbitan monopalmitate) and aqueous dispersions of carotene prepared without the surface-active agent. Such last dispersions also of vitamin A were given to rabbits.

2. Carotene in oily solution or dispersed in Tween was given by mouth to the three kinds of animal, and vitamin A in oil to calves and rabbits.

3. Carotene in Tween dispersion, injected into the blood of rats and rabbits, had disappeared from it almost completely after 24 h. Carcass analysis of rabbits at different times after injection showed that the carotene disappearing from the blood was largely destroyed. Within 5 min of the injection vitamin A alcohol appeared in the blood of rats and increased in that of rabbits and within half an hour deposits of vitamin A alcohol appeared in the liver of rats.

4. The form of vitamin A and the time of its appearance provided evidence that the injected carotene was converted to vitamin A elsewhere than in the intestinal wall.

5. Carotene in aqueous dispersion without Tween could not be accounted for quantitatively in the blood of rats or rabbits; it disappeared from the blood with great rapidity and did not give rise to vitamin A.

6. Whatever the form of dispersion, carotene injected into calves was converted, if at all, only to a very limited extent into vitamin A.

7. Only half of the dose of carotene injected in Tween ever appeared in the blood of calves but carotene persisted in the blood for up to 10 days. Carotene in aqueous dispersion without Tween appeared in small quantities in the blood only some 3-6 h after the injection, being presumably liberated from a site of primary deposition.

8. Whatever the dispersion or the animal, injected vitamin A appeared in the blood as the alcohol but never quantitatively. In rats half of the dose was deposited in the liver but in calves only one-tenth of it.

9. In the rabbit and calf appreciable but rapidly diminishing quantities of injected vitamin A or carotene appeared in the lungs. No information on this point was obtained for rats.

10. In all three species carotene given by mouth was better absorbed and more efficiently converted into vitamin A from Tween dispersion than from oily solution.

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Thyroxine, Stilboestrol and Antibiotics in Rations for Castrated Male Pigs

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The experiment reported in this paper was one of a series undertaken by various research stations on behalf of the U.K. Agricultural Research Council and co-ordinated by Dr R. Braude. A summary of all these experiments has been published (Braude, Campbell, Lucas, Luscombe, Robinson & Taylor, 1955), but it was impracticable to include in this summary all details of results obtained and opinions expressed by each research station, and we give our results in more detail in this report.

Braude (1950) described four experiments on feeding iodinated casein and stilboestrol to castrated male pigs. The results were more promising than in former experiments when iodinated casein and stilboestrol were fed separately. Later Barber, Braude & Mitchell (1953) reported that the inclusion of 0.3 mg L-thyroxine pentahydrate and 6 mg stilboestrol/lb. meal resulted in a 6.4% increase in growth rate over that of unsupplemented controls. A supplement of 5 lb. Aurofac 2A (Lederle Laboratories Division, Cyanamid Products Ltd, London) per ton of meal resulted in a 12.0% increase in growth rate, but when L-thyroxine pentahydrate and stilboestrol and Aurofac were all included in the same ration the positive growth response was 21.2%, with a 10% advantage in food conversion efficiency. The objects of the experiment reported here were:

(1) To confirm the observation that a combination of thyroxine, stilboestrol and aureomycin in rations for castrated male pigs produces a growth response considerably greater than that induced by aureomycin alone.

(2) To extend the observations by studying the effects of rations containing procaine penicillin and procaine penicillin with thyroxine and stilboestrol.

EXPERIMENTAL

Housing. Six wooden ark huts were used, each having a small run on concrete. Each unit of hut and run was fitted with eight individual feeding compartments.