pregnant animals declined during the same period. In all the animals no major changes took place in the quantitative relationships of the different classes of plasma lipid (cholesterol esters, triglycerides, phospholipids and free fatty acids), though the small proportion of fatty acids contributed by the triglycerides to the plasma total fatty acids declined progressively during pregnancy to parturition and then further during lactation.

3. Though pregnancy, parturition and lactation were not accompanied by any significant changes in the fatty acid composition of the plasma glycerides, phospholipids and free fatty acids, the plasma cholesterol esters during pregnancy contained a much greater proportion of C_{18} di-unsaturated acid relative to that of C_{18} tri-unsaturated acid than was present at parturition and during lactation.

4. 3,7,11,15-Tetramethylhexadecanoic acid was found in the plasma lipids (particularly triglycerides and phospholipids) of all the animals.

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Studies on the Mode of Action of Excess of Vitamin A

8. MITOCHONDRIAL SWELLING*

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Many of the changes seen in hypervitaminosis A are explicable on the hypothesis that vitamin A penetrates lipoprotein membranes and that membranes containing an excess of the vitamin exhibit increased permeability or decreased stability. Model experiments with monolayers of lecithin and cholesterol on a Langmuir trough have shown that vitamin A alcohol readily penetrates lipid films and produces an increase of surface area (Bangham,

Dingle & Lucy, 1964). The instability that results from penetration by an excess of vitamin A is exemplified by the rapid haemolysis of erythrocytes that occurs on treatment with the vitamin (Dingle & Lucy, 1962). Phase-contrast-microscopic and electron-microscopic studies have shown that this haemolysis is preceded by drastic changes in the structure of erythrocytes which include buckling of the cells and the formation of vacuoles by a process that resembles micropinocytosis (Glauert, Daniel, Lucy & Dingle, 1963). In experiments on the effect of excess of vitamin A on fibroblasts, disruption of the cell membranes and nuclear membranes and swelling

* Part 7: Glauert, Daniel, Lucy & Dingle (1963).

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of mitochondria were observed (Dingle, Glauert, Daniel & Lucy, 1962). Alterations in mitochondrial structure may lead to disturbances of respiration such as the depression of oxygen uptake in embryonic chick limb-bone rudiments treated with vitamin A *in vitro* (Dingle, Lucy & Fell, 1961). Also, vitamin A depresses the oxygen uptake of isolated mitochondria in the presence of hexokinase and glucose (H. F. DeLuca & J. T. Dingle, unpublished work).

These several observations indicated that penetration of the lipoprotein membrane of the isolated mitochondrion by the vitamin might result in the swelling of this organelle; results reported in the present paper show that vitamin A causes swelling of isolated mitochondria similar to that produced by thyroxine and by Fe^{2+} ions. The molecular specificity for the production of this effect by compounds in the vitamin A series is related to that for the prevention of vitamin A deficiency. These observations support the general hypothesis concerning the mode of action of excess of vitamin A that has been advanced on the basis of previous experiments done in this Laboratory (Fell & Thomas, 1960; Lucy, Dingle & Fell, 1961; Dingle, 1961; Dingle & Lucy, 1962; Lucy & Dingle, 1962).

MATERIALS AND METHODS

Vitamin A derivatives. Vitamin A alcohol (Roche Products Ltd.) was dissolved in ethanol to give a final concentration of 100 mg./ml. This solution was stored under nitrogen in the cold and was diluted with ethanol immediately before use. 4-Apo- β -carotenol and 3-apo- β -carotenol were given by Dr J. Glover and Professor R. A. Morton, F.R.S., of the University of Liverpool. The methyl ether of vitamin A, β -ionylidene-ethanol, β -ionylidene-acetaldehyde, vitamin A₂ alcohol and vitamin A₂ aldehyde were gifts from Dr P. L. Harris of the Biochemistry Laboratories, Distillation Products, Rochester, N.Y., U.S.A. Other derivatives of vitamin A were prepared and stored as described by Fell, Dingle & Webb (1962) and by Dingle & Lucy (1962).

Preparation of mitochondria. Tissues were obtained from adult rats and were cooled immediately after removal from the animal by immersion in ice-cold 0.4 M-sucrose buffered with 0.05 M-tris, pH 7.4. Tissue samples were then weighed and a 1:10 (w/v) suspension was made, a Perspex motor-driven homogenizer being used (Aldridge, Emery & Street, 1960). The suspension was centrifuged at 600 g for 5 min. to remove cell debris and nuclei, and the supernatant was then spun at 10 000 g for 20 min. in an angle-head M.S.E. centrifuge. The 10 000 g pellet was resuspended by gentle homogenization in a glass Potter homogenizer and respun at 10 000 g for 10 min. This operation was repeated twice more. The washed mitochondrial pellet was then resuspended in 10 ml. of 0.4 M-sucrose-0.05 M-tris buffer, pH 7.4, or in 0.125 M-KCl-0.02 M-tris buffer, pH 7.4, and diluted about 1:25 with suspending medium to give an extinction at 500 m μ of approx. 0.5. Experiments on the temperature-dependence of the vitamin's effect were made in unbuffered

0.4 M-sucrose. All preparatory work was done in a cold room at a temperature of approx. 4°.

Measurement of mitochondrial swelling. Mitochondrial swelling was measured by the change in extinction at 500 m μ in a manner similar to that described by Lehninger, Ray & Schneider (1959). A Unicam SP. 500 spectrophotometer with a water-jacketed cell-housing to control the temperature was used; the mitochondrial suspension was warmed to the required temperature just before transfer to the cell-housing. A sample (0.03 ml.) of an ethanolic solution of vitamin A or other compounds was added to a dry cuvette and, after the addition of 3 ml. of the suspension, readings were taken every 20 sec. for 5 min. In some experiments a Unicam SP. 700 recording spectrophotometer was used. The change in extinction after treatment with vitamin A was associated with an increase in the water content of the mitochondria.

Uptake of vitamin A by mitochondria. Mitochondria suspended in sucrose-tris buffer, pH 7.4, were treated with vitamin A alcohol, vitamin A acetate and vitamin A aldehyde in a manner similar to that in which swelling was measured. After 5 min. at room temperature (approx. 21°) the suspension was spun at 10 000 g for 10 min., and washed by resuspending in sucrose-tris buffer and re-spinning. The pellet was resuspended in sucrose-tris buffer, and an equal volume of ethanol was added; the mixture was shaken and 2 vol. of light petroleum (b.p. 40–60°) added. After extraction, the aqueous phase was re-extracted with 1 vol. of light petroleum. The light-petroleum extract was evaporated to dryness under nitrogen and the extracted material was redissolved in ethanol. The ultraviolet-absorption spectrum was determined with a Unicam SP. 700 recording spectrophotometer, and the amount of vitamin recovered determined from the extinction coefficient at the maximum absorption.

RESULTS

Action of vitamin A on mitochondria. Under the conditions of these experiments the addition of ethanol (1%, v/v) to the mitochondrial suspension had no effect. The addition of vitamin A (10 $\mu\text{g.}/\text{ml.}$) dissolved in ethanol, however, caused a rapid decrease in extinction (Fig. 1). At a vitamin concentration of 10 $\mu\text{g.}/\text{ml.}$ and at a temperature of 29°, the swelling was most rapid during the first 2½ min. In the light of this observation, the effect of various concentrations of vitamin A was investigated during the first few minutes; Table 1 records the swelling produced by concentrations of the vitamin of between 2 and 10 $\mu\text{g.}/\text{ml.}$ during 40 and 60 sec. of incubation at 37°. The concentration for activity under these conditions, i.e. approx. 10 μM , was very similar to that found for thyroxine by Lehninger *et al.* (1959) and for sodium oleate by Wojtczak & Lehninger (1961). For most experiments, 10 $\mu\text{g.}$ of vitamin A/ml. (35 μM) was chosen as a convenient concentration.

Effect of temperature. The action of the vitamin on a subcellular fraction containing lysosomes (Dingle, 1961) and on suspensions of erythrocytes

(Dingle & Lucy, 1962) has been shown to be strongly dependent on temperature. This is also true of its effect on isolated mitochondria. For instance, although 10 μ g. of vitamin A/ml. caused a change in extinction of about 0.2 at 37° in 2 min., the change at 3° during the same time was only 0.01. The effect of altering the temperature of incubation on the action of the vitamin is shown in Fig. 2. At temperatures above 20° there was a slight effect of ethanol, but this was small compared with the action of vitamin A. The dependence of swelling produced by vitamin A on temperature was almost linear during the first minute.

Molecular specificity. Table 2 shows the results of a comparative study of the activity of vitamin A alcohol, vitamin A₂ alcohol, vitamin A acid and many other derivatives of vitamin A. The only

compound with a comparable activity to that of vitamin A₁ alcohol was vitamin A₂ alcohol. The esters of vitamin A, the dimethyl ether of the vitamin and vitamin A aldehyde were all inactive. Increasing the length of the side chain of the molecule greatly interfered with the ability of derivatives

Fig. 1. Vitamin A-induced swelling of rat-liver mitochondria. A suspension of mitochondria in 0.4M-sucrose was treated with vitamin A alcohol (10 μ g./ml.) at 29° and the change in extinction at 500 m μ measured for 5 min. ●, Control; ○, 10 μ g. of vitamin A alcohol.

Table 1. *Effect of vitamin A on mitochondrial swelling*

Rat-liver mitochondria were suspended in 0.4M-sucrose and incubated at 37°. The extinction at 500 m μ was measured after the addition of an ethanolic solution of vitamin A alcohol. The final concentration of ethanol was 1.0% (v/v).

Concn. of vitamin A (μ g./ml.)	Change in $E_{500\text{ m}\mu}$	
	After 40 sec.	After 60 sec.
0	0	0
2.0	0.005	0.007
5.0	0.035	0.083
7.5	0.095	0.156
10.0	0.145	0.177

Fig. 2. Effect of temperature on vitamin A-induced swelling of rat-liver mitochondria. A suspension of mitochondria in 0.4M-sucrose was treated with vitamin A alcohol (10g./ml.) and the decrease in extinction at 500 m μ measured after incubation at various temperatures for 1 and 2 min. ○, After 2 min. of incubation with vitamin A (alcohol); ●, after 1 min. of incubation with vitamin A (alcohol); ▲, after 2 min. of incubation with ethanol control.

Table 2. *Effect of various compounds on isolated rat-liver mitochondria*

Mitochondria were suspended in 0.4M-sucrose containing tris buffer, pH 7.4 (0.05M). All compounds were tested at a concentration of 35 μ M. Results are expressed as percentages of the change in extinction at 500 m μ produced in 2 min. by 35 μ M-vitamin A alcohol at 37°.

Temperature ...	Change in $E_{500\text{ m}\mu}$ (%)		
	3°	19°	37°
Vitamin A alcohol	10	80	100
Vitamin A acid	0	—	22
Vitamin A aldehyde	—	10	20
Vitamin A ₂ alcohol	13	120	148
Vitamin A ₂ aldehyde	0	26	16
Vitamin A methyl ether	0	0	0
Vitamin A palmitate	0	0	0
Vitamin A acetate	0	0	0
Anhydro-vitamin A	0	0	0
Hydrogenated vitamin A	4	8	12
β -Ionylidene-ethanol	0	—	19
β -Ionylidene-acetaldehyde	0	11	0
3-Apo- β -carotenol	0	0	0
3-Apo- β -carotenal	0	0	0
4-Apo- β -carotenol	0	16	52
4-Apo- β -carotenal	0	0	0
Geraniol	0	0	—
Citral	0	0	—
β -Ionone	0	0	0
Ethanol	0	0	0

Table 3. *Effect of various compounds on mitochondria isolated from different tissues of the rat*

Mitochondria were suspended in 0.4 M-sucrose containing tris buffer, pH 7.4 (0.05 M), and incubated for 5 min. at 19°. All compounds were tested at a concentration of 35 μ M. Results are expressed as percentage changes in extinction at 500 m μ (the initial extinction was 0.5).

	Change in $E_{500 \text{ m}\mu}$ (%)				
	Liver mitochondria	Kidney mitochondria	Heart mitochondria	Spleen mitochondria	Brain mitochondria
Ethanol	0	0	0	0	0
Vitamin A alcohol	31	17	8	2	2
Vitamin A ₂ alcohol	28	20	15	—	—
Vitamin A acid	16	3	4	—	—
Vitamin A aldehyde	5	—	—	0	0
β -Ionylidene-acetaldehyde	7	0	—	—	—
L-Thyroxine* (30 μ M)	30	12	2	0	4

* Results of Tapley & Cooper (1956).

Fig. 3. Inhibitory action of bovine plasma albumin on the swelling of mitochondria induced by vitamin A alcohol. Mitochondria were suspended in 0.4 M-sucrose–0.05 M-tris buffer, pH 7.4, containing bovine plasma albumin (fraction V) at various concentrations. Vitamin A alcohol (10 μ g./ml.) was added and the decrease in extinction at 500 m μ measured after 5 min. of incubation at 37°. Results are expressed as percentages of the decrease in extinction given by vitamin A-treated mitochondria.

of vitamin A to cause mitochondrial swelling. Derivatives that were inactive at 37° were also inactive in the cold.

Tissue specificity. A number of compounds were tested on mitochondria isolated from rat liver, kidney, heart, spleen and brain. Vitamin A alcohol was again the most effective compound of those tested (Table 3). Comparison of the action of vitamin A with that reported for thyroxine by Tapley & Cooper (1956) showed a similar tissue specificity. Thus mitochondria from liver were the most readily swollen by both vitamin A and thyroxine, and those from spleen and brain were the least affected by these agents.

Inhibition of mitochondrial swelling by protein.

Since bovine plasma albumin (fraction V) inhibits the haemolysis of rabbit erythrocytes by vitamin A (Dingle & Lucy, 1962), the inhibitory action of protein on mitochondrial swelling induced by the vitamin was investigated. Fig. 3 shows that the presence of increasing quantities of albumin caused progressively greater inhibition of the swelling; thus 4.0 mg. of albumin/ml. inhibited approx. 80 % of the swelling caused by 10 μ g. of vitamin A/ml. This action of protein is probably related to its ability to bind the vitamin and emphasizes the need to use adequately washed mitochondria if reproducible results are to be obtained.

Effect of cyanide on mitochondrial swelling induced by vitamin A. Rat-liver mitochondria show a greater response to vitamin A alcohol and vitamin A aldehyde when suspended in potassium chloride–tris buffer than when sucrose–tris buffer is used (Table 4). Although the swelling action of a variety of agents is inhibited by potassium cyanide (Lehninger & Ray, 1957; Chappell & Greville, 1958), cyanide does not inhibit swelling caused by 17 μ M-vitamin A.

Uptake of vitamin A by mitochondria in vitro. Treatment of a mitochondrial suspension (initial extinction 0.6 at 500 m μ) with vitamin A alcohol, vitamin A acetate and vitamin A aldehyde (all at 5 μ g./ml.) gave recoveries of 2.9, 1.4 and 1.0 μ g./ml. respectively. Since the ultraviolet-absorption spectra of the recovered compounds did not differ appreciably from that of the pure vitamins used, it is unlikely that there was any significant change in the structure of the molecules during their brief contact with the mitochondria. This experiment does not differentiate, however, between vitamin molecules that have penetrated the membrane and those that are adsorbed on to the surface of the particles. Nevertheless, it may be relevant that the recoveries of the three compounds were proportional to their relative effectiveness in causing mitochondrial swelling.

Table 4. *Effect of various compounds on mitochondria suspended in different media*

Rat-liver mitochondria were prepared in 0.4M-sucrose-0.1M-tris buffer, pH 7.4, and then suspended in the media indicated to give an extinction of 0.6 at 500 m μ . After the addition of the vitamin (final concentration 17 μ M) the change in extinction at 500 m μ was measured on a recording instrument for 3 min. at 21°.

Suspending medium	Change in $E_{500\text{ m}\mu}$			
	Vitamin A alcohol	Vitamin A aldehyde	Vitamin A acetate	Ethanol
0.4M-Sucrose-0.1M-tris, pH 7.4	0.27	0.08	0.08	0
0.125M-KCl-0.02M-tris, pH 7.4	0.39	0.16	0.02	0
0.125M-KCl-0.02M-tris buffer, pH 7.4, containing KCN (1 mM)	0.41	—	—	0
0.125M-KCl-0.02M-tris buffer, pH 7.4, containing KCN (3 mM)	0.43	—	—	0

DISCUSSION

Results reported in the present paper demonstrate that vitamin A, at concentrations comparable with those of thyroxine and sodium oleate, causes rapid changes in mitochondria isolated from several different tissues. The dose response, temperature-dependence and molecular-specificity requirements for activity are essentially similar to those reported for the action of vitamin A in other systems (cf. Dingle & Lucy, 1962). The reason for the relative ineffectiveness of the vitamin on mitochondrial particles isolated from brain, spleen and heart as compared with its action on liver mitochondria is unknown. It is possible that these results reflect differences in the lipoprotein membranes of mitochondria from various tissues and hence a difference in the ability of vitamin A to penetrate these membranes. The comparative inactivity of the vitamin on kidney mitochondria may be related to the findings of Dingle, Sharman & Moore (1963), who observed that lysosomes from the liver of hypervitaminotic animals appeared to be much more unstable than those from control animals, whereas lysosomes obtained from the kidney showed only slight alterations in stability.

Since the publication of the initial report from this Laboratory on the swelling action of vitamin A on mitochondria *in vivo* and *in vitro* (Dingle *et al.* 1962), Wang, Slater & Dartnall (1963) have confirmed our observations on the action of the vitamin on isolated liver mitochondria. Further, under their experimental conditions Wang *et al.* (1963) could find no action of vitamin A on mitochondria isolated from cattle retinae.

The different reactions of mitochondria from different tissues towards treatment with vitamin A are similar to those produced by thyroxine on mitochondria isolated from the same tissues (Tapley & Cooper, 1956). Swelling induced by thyroxine also resembles that produced by vitamin A in having a high temperature coefficient, and in occurring more readily in a potassium chloride medium than in a sucrose medium (Lehninger *et al.* 1959). Further, both thyroxine and vitamin A are

inhibited by serum albumin. The inhibitory action of albumin on thyroxine-induced swelling is due to trapping by the protein of iso-octane-soluble material designated 'U factor' (Wojtczak & Lehninger, 1961). This factor, which appears to be a mixture of saturated and unsaturated fatty acids, is formed in mitochondria under the influence of thyroxine. Serum albumin is able to bind U factor and vitamin A, and thus prevent mitochondrial swelling that is otherwise produced by each of these agents. It is possible that the mechanisms whereby these lipophilic compounds penetrate and expand or disrupt the mitochondrial membrane are similar.

The inactivity of cyanide on the swelling of vitamin A-treated mitochondria suggests that the action of the vitamin, unlike that of thyroxine and similar agents, is not dependent on respiration. The swelling induced by vitamin A more closely resembles that caused by Fe²⁺ ions than by thyroxine, since 1 mM-sodium cyanide produces only partial inhibition (10–50%) of the swelling induced by Fe²⁺ ions (Hunter, Gebicki, Hoffsten, Weinstein & Scott, 1963). It has been shown that swelling of mitochondria caused by Fe²⁺ ions is so slow in 0.33M-sucrose as to be negligible; potassium chloride is the most satisfactory medium for studying the phenomenon. Hunter *et al.* (1963) have also demonstrated that there is a close correlation between lipid peroxide formation and swelling or lysis induced by Fe²⁺ ions. Antioxidants, including α -tocopherol, produced nearly complete inhibition of lipid peroxidation and swelling caused by 50 μ M-Fe²⁺ ion. J. A. Lucy & J. T. Dingle (unpublished work) have shown that mitochondrial swelling caused by vitamin A alcohol is inhibited by α -tocopherol acetate. The rapid haemolytic action of vitamin A alcohol is also inhibited by α -tocopherol acetate, and it has been proposed that oxidation of excess of vitamin A alcohol within the membrane of the erythrocyte is a primary factor in the rapid release of haemoglobin that occurs in the absence of vitamin E (Dingle & Lucy, 1963). Since cyanide, which under certain circumstances acts as an antioxidant, is without effect on mitochondrial swelling caused by vitamin A

alcohol, it is not clear to what extent oxidation is concerned in vitamin A-induced swelling.

The swelling of mitochondria in response to vitamin A is not limited to conditions *in vitro* but may also occur in the living cell. Thus electron-microscopic studies of fibroblasts suspended in Tyrode's solution and treated with vitamin A showed that changes in the cell membranes and nuclear membranes occurred and that the intracellular organelles became swollen and distorted (Dingle *et al.* 1962). Further, Jackson & Fell (1963) have cultivated embryonic chicken skin in the presence of vitamin A and demonstrated mitochondrial swelling in the cells of the epidermis. The effect of vitamin A on mitochondrial structure may be related to its action on lysosomes, which, when exposed to the vitamin, release proteolytic enzymes both *in vitro* and *in vivo* (Dingle, 1961; Fell & Dingle, 1963).

In causing mitochondrial swelling, as in its other effects, quite small changes in the molecular structure of vitamin A cause marked differences in its activity. Only vitamin A₂ alcohol was as effective as vitamin A₁ alcohol in producing swelling. Vitamin A₂ alcohol was, however, less active in lysing erythrocytes (Dingle & Lucy, 1962). In general, the molecular specificity for the production of mitochondrial swelling was similar to that required for the action of excess of vitamin A on isolated lysosomes, on embryonic cartilage *in vitro* and on isolated erythrocytes. It is thought that the common factor between these systems is to be found in the ability of the vitamin to penetrate lipoprotein membranes.

In any attempt to relate studies on the action of excess of vitamin A *in vitro* to the biological role of the vitamin, consideration must be given to the fact that, although a large quantity of vitamin A is stored in the liver, it has no ill effect on the cells or their organelles. The probable explanation of this apparent paradox is that the vitamin is stored mainly as an ester, and esters of vitamin A have been found to be inactive in penetrating lipid films, in haemolysing erythrocytes and in releasing lysosomal protease. As suggested by Dingle & Lucy (1962), it is probable that the activity of the ester *in vivo* is due to its hydrolysis by an appropriate esterase.

The presence of vitamin A in mitochondria has been reported by several workers, although this has been disputed by Krinsky & Ganguly (1953). If the vitamin is normally present in mitochondrial membranes, absence of the vitamin in deficiency conditions, as well as the presence of excess in hypervitaminosis, might be expected to lead to distortion of mitochondria structure. Abnormally swollen mitochondria have, in fact, been obtained from the tissues of animals suffering from vitamin A

deficiency (H. F. DeLuca, personal communication), and Sheldon & Zetterqvist (1955) described changes in mitochondrial morphology in the keratinizing epithelia of vitamin A-deficient mice. Though absence of vitamin A may impair the structure and possibly the normal functions of the mitochondrion, excess of the vitamin causes swelling that apparently stems initially from penetration of the mitochondrial membrane by the excess of vitamin A. It is suggested that one important role of the vitamin is that its presence in optimum quantities may be necessary for the maintenance of the normal structure and functioning of mitochondria: changes in structure and function may result from either decreased or increased concentrations of vitamin A.

SUMMARY

1. Vitamin A alcohol, at a concentration of 35 μM , causes rat-liver mitochondria to swell rapidly; swelling occurs more readily in a potassium chloride medium than in a sucrose medium.

2. The swelling is temperature-dependent, and the vitamin has little action on mitochondria in the cold.

3. Vitamin A alcohol has less effect on mitochondria from rat spleen and brain than on mitochondria from rat liver and kidney.

4. Bovine plasma albumin (fraction V) inhibits the action of vitamin A alcohol on rat-liver mitochondria; potassium cyanide (3 mM) is ineffective in inhibiting mitochondrial swelling caused by the vitamin.

5. Vitamin A₁ alcohol and vitamin A₂ alcohol are the only compounds of those tested in the vitamin A series that cause rapid mitochondrial swelling at a concentration of 35 μM .

6. The ability of vitamin A to cause mitochondrial swelling is thought to be consistent with the hypothesis that one site of action of the vitamin is within membranes; it is suggested that the presence of the vitamin in optimum quantities may be required for the maintenance of the normal structure and functioning of mitochondria.

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Inhibition of a Mammalian Deoxyribonucleic Acid Nucleotidyltransferase by Actinomycin D

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The isolation of Actinomycin from a culture of *Streptomyces antibioticus* was reported by Waksman & Woodruff (1940). Various forms of Actinomycin have since been described, one of which, Actinomycin D (Vining & Waksman, 1954), is an orange antibiotic consisting of two pentapeptide chains covalently bound to a chromophoric phenoxazine derivative (Brockmann, 1960). It is a powerful inhibitor of the growth of mammalian cells in tissue culture and its action has been reported by Reich, Franklin, Shatkin & Tatum (1962*a*) to be due to selective and complete inhibition of cellular RNA synthesis. Further work (Reich, Goldberg & Rabinowitz, 1962*b*; Goldberg, Rabinowitz & Reich, 1962) revealed that the site of inhibition was the DNA-dependent RNA-nucleotidyltransferase system (nucleoside triphosphate-ribonucleic acid nucleotidyltransferase, EC 2.7.7.6) and that the Actinomycin D exerted its effect specifically by binding to the guanine residues of the DNA-primer.

Kirk (1960) observed that the antibiotic inhibited the activity of DNA nucleotidyltransferase (deoxynucleoside triphosphate-deoxyribonucleic acid deoxynucleotidyltransferase, EC 2.7.7.7) from *Escherichia coli*, and Hurwitz, Furth, Malamy & Alexander (1962), using highly purified enzyme preparations from *E. coli*, showed that the inhibition of both DNA and RNA nucleotidyltransferases

obeyed competitive kinetics with respect to the DNA-primer.

However, no information has yet appeared on the action of Actinomycin D in soluble DNA-synthesizing systems derived from mammalian cells. Keir, Binnie & Smellie (1962) and Keir (1962) have described a partially purified preparation of DNA nucleotidyltransferase from Landschutz ascites-tumour cells, and in the present paper we present evidence indicating that the activity of this enzyme fraction is inhibited competitively by Actinomycin D.

EXPERIMENTAL

Abbreviations. The 5'-triphosphates of deoxyadenosine, deoxycytidine and deoxyguanosine, deATP, deCTP and deGTP respectively; thymidine 5'-triphosphate labelled with ³²P in the α-phosphate group, [³²P]TTP; thymidine 5'-monophosphate labelled with ³²P, [³²P]TMP; deoxyribonuclease active at about pH 7.5 in the presence of Mg²⁺ ions and producing 5'-phosphoryl-terminal oligonucleotides, deoxyribonuclease I.

Preparation of enzyme fraction. The enzyme fraction used in these experiments was derived from cells of the Landschutz ascites tumour of the mouse, and its preparation and some of its properties with respect to DNA nucleotidyltransferase and deoxyribonuclease I have been fully described by Keir (1962).