Isolation of Chick Intestinal Nuclei

EFFECT OF VITAMIN D₃ ON NUCLEAR METABOLISM

By D. E. M. LAWSON, P. W. WILSON, D. C. BARKER AND E. KODICEK

*Dunn Nutritional Laboratory, University of Cambridge and
Medical Research Council, Cambridge CB4 1XJ*

(Received 16 June 1969)

1. Chick intestinal nuclei were isolated, with practically no contamination from other organelles and whole cells, by centrifugation through 2.4M-sucrose. 2. The proportions of RNA, DNA and protein of the isolated nuclei were unaffected by the vitamin D status of the birds. The RNA/DNA ratio was 0.15. 3. The incorporation of [5-³H]orotic acid into the rapidly labelled intestinal nuclear RNA, after a 10 min. pulse of the orotic acid, was increased in vitamin D-deficient chicks only 10 min. after a 125 µg. dose of cholecalciferol. 4. There was no stimulation of the DNA-dependent RNA polymerase activity of the isolated nuclei from birds treated with cholecalciferol. 5. The results are discussed in relation to the changes occurring during the lag period, after administration of cholecalciferol and before Ca²⁺ transport is detected, and the function of the vitamin.

One aspect of the attempts to understand the mode of action of vitamin D has been the studies carried out into its subcellular distribution. Originally there was general agreement that the highest concentration of vitamin D in the intestine of the deficient animal occurred in the microsomal fraction (Kodicek, 1963; Norman & DeLuca, 1964). However, subsequent publications from a number of laboratories have focused attention on the nucleus as a possible site of action of the vitamin. Haussler & Norman (1967) and Stohs & DeLuca (1967) have reported that, with a very low dose of [1,2-³H]-cholecalciferol, the highest proportion was found in the nuclear fraction of chick intestine, and Wilson, Lawson & Kodicek (1967) found the highest concentrations of [1α-³H]cholecalciferol, after an oral dose of the vitamin, to be in a fraction of rat intestine containing at least nuclei and brush border. Eisenstein & Passavoy (1964) reported that the vitamin D-mediated stimulation of Ca²⁺ transport across the intestine could be inhibited *in vivo* by the administration of actinomycin D 1 or 2 hr. before the vitamin, suggesting either that the action of vitamin D is dependent on the occurrence of continual protein synthesis, or that it is involved in the elaboration of the protein, possibly at the stage of transcription of the nucleic acids.

As neither Haussler & Norman (1967) nor Stohs & DeLuca (1967) reported on the purity of their nuclei it seemed that investigations into the function of vitamin D would be aided by the isolation of an intestinal nuclear fraction of accepted purity.

However, the fractionation of mucosal homogenates from animal intestines, particularly the rat, is difficult, owing to the presence of mucus. The most systematic studies of the subcellular fractionation of mucosa have been carried out by Porteous & Clark (1965) and Hübscher, West & Brindley (1965), but neither group reported obtaining pure nuclei. We have applied the method of Widnell & Tata (1964) for the isolation of liver nuclei to this problem, but were successful only with chick and not rat intestine. With the pure nuclei so obtained from chick intestine the effect of vitamin D on the DNA-dependent RNA polymerase activity and the incorporation of [5-³H]orotic acid into nRNA* was investigated. The metabolic fate of labelled cholecalciferol is described in the next paper (Lawson, Wilson & Kodicek, 1969), in which the presence of a new metabolite in cell nuclei is reported.

EXPERIMENTAL

Animals. The care and maintenance of the rats has been described (Wilson *et al.* 1967). Chunky chicks (1 day old) were obtained from Sterling Poultry Services Ltd. (Welwyn Garden City, Herts.). They were fed on the following diet *ad lib.*: ground yellow corn, 54%; ground whole wheat, 20%; ground rolled oats, 10%; crude acid-precipitated casein, 11.5%; CaCO₃, 1%; calcium phosphate, 0.5%; iodized salt, 1%; MnSO₄, 4H₂O, 0.02%; non-irradiated

* Abbreviation: nRNA, nuclear RNA.

yeast, 2%. Supplements of vitamins A, E and K were given weekly. After about 3 weeks on this diet the birds were vitamin D-deficient. The dose of cholecalciferol (vitamin D₃) was administered intracardially to each animal in 0.2 ml. of propylene glycol. The animals were kept without food for 16 hr. before being killed by cervical dislocation. The small intestine was quickly removed, washed through with ice-cold 0.9% NaCl, opened longitudinally, rinsed with cold 0.9% NaCl and the mucosa was scraped off.

Isolation of nuclei. (a) Nuclei for chemical and enzymic studies were isolated by the procedure of Widnell & Tata (1964), with Mg²⁺ as the bivalent cation. This is necessary, as Ca²⁺ is inhibitory of RNA polymerase (Weiss, 1960). For electron-microscopic examination of the isolated nuclei the Mg²⁺ was replaced with 1 mM-Ca²⁺ throughout the procedure. The nuclei were resuspended in 0.25 M-sucrose with the addition of MgCl₂ (final concn. 1 mM).

(b) The yield of nuclei from intestinal mucosa obtained by the above procedure was low, although the nuclei were virtually uncontaminated by any other cell fraction. We subsequently found that a higher yield (45%) of pure nuclei of similar purity and with intact membranes could be obtained by the following method.

The mucosa was finely minced with scissors, and then homogenized in 4 vol. of 0.32 M-sucrose in TKM buffer [50 mM-tris-HCl (pH 7.5)-25 mM-KCl-5 mM-MgCl₂] in a Potter-Elvehjem Teflon-to-glass homogenizer (radius clearance of 0.003-0.005 in.) with six to ten strokes by hand. The homogenate was diluted with an equal volume of 0.32 M-sucrose in TKM buffer and filtered through nylon bolting cloth (St Martins 10N). The resulting suspension was centrifuged for 10 min. at 800g and the nuclear-debris pellet was resuspended and washed three times with an equal volume of 0.32 M-sucrose in TKM buffer. The final pellet was resuspended into a volume of 0.32 M-sucrose in TKM buffer (1 ml./g. of original mucosa), and 8 vol. of 2.4 M-sucrose-1 mM-CaCl₂ were added very slowly with continuous mixing to prevent any rapid rise in molarity. This suspension was centrifuged for 1 hr. at 53 500g in a Spinco SW25 swing-out rotor. A white pellet of packed nuclei was obtained.

All procedures were carried out at 4°.

Extraction of rRNA. A portion of the intestinal mucosa (1 g.) or the isolated intestinal nuclei obtained from chicks that had received an intraperitoneal injection of [5-³H]-orotic acid were homogenized in 5 ml. of 0.5% sodium dodecyl sulphate in the presence of 5 mg. of bentonite for 1 min. in an MSE model 7700 homogenizer with a 10 ml. container. The solution was then shaken with an equal volume of water-saturated phenol for 1 hr. and centrifuged at 9000g. The aqueous layer was removed and the phenol layer washed three times with water. The combined aqueous extracts were made 2% with respect to sodium acetate, and 2.5 vol. of ethanol was added to precipitate the RNA, which was washed successively with ethanol, acetone and ether. The RNA was then hydrolysed with 0.5 M-NaOH (1 ml./20 mg. of precipitate). The solution was neutralized with 5 M-HCl and samples were taken for measurement of radioactivity and u.v. analysis in 0.01 M-HCl.

Electron microscopy. The intestinal mucosa and nuclear pellets were fixed for 16 hr. at 4° in 6.25% (w/v) glutaraldehyde in 0.88 M-sucrose. During fixation the mucosa was

cut into small blocks of less than 1 mm.³. The specimens were washed in several changes of 0.88 M-sucrose and post-fixed in 1% OsO₄ in 0.1 M-phosphate buffer, pH 7.6, for 16 hr. All fixing and wash solutions contained 1 mM-CaCl₂. The pellets were dehydrated in a graded series of cold ethanol and stained in the block with a saturated solution of uranyl acetate in 70% ethanol. Pellets were embedded in Epikote 812 mixture adjusted to give a hard block. Sections showing silver or gold interference colours were cut with glass or diamond knives on a Reichart OMU2 or a Cambridge Huxley microtome. The sections were mounted on collodion carbon-coated grids and stained with 6% uranyl acetate for 10-30 min. Micrographs were taken on an AEI electron microscope at 60 kv with a 50 μm. objective aperture and liquid-nitrogen decontaminator and instrumental magnification of 5000-60 000.

Enzyme assay. For DNA-dependent ribonucleoside triphosphate-ribonucleic acid nucleotidyltransferase (RNA polymerase; EC 2.7.7.6), the Mg²⁺-stimulated and Mn²⁺-stimulated reactions were measured. The Mg²⁺-stimulated RNA polymerase reaction was assayed by the procedure of Widnell & Tata (1964), with tris buffer, pH 8.5. The Mn²⁺-stimulated reaction was assayed by the procedure of Tata & Widnell (1966).

Cytochrome oxidase (EC 1.9.3.1) activity was assayed by the method of Smith (1955).

Invertase (EC 3.2.1.26) activity was assayed by the method of Carnie & Porteous (1962).

Glucose 6-phosphatase (EC 3.1.3.9) was assayed as described by Swanson (1955).

6-Phosphogluconate dehydrogenase (EC 1.1.1.44) was assayed essentially by the method of Glock & McLean (1953).

Chemical determinations. Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard. RNA and DNA were extracted from tissue and cell fractions as described by Schneider (1957). The determinations of RNA and DNA were carried out by the methods of Ceriotti (1955) and Burton (1956) respectively, with yeast RNA and calf thymus DNA as standards.

Measurement of radioactivity. These were made in Packard Tri-Carb automatic liquid-scintillation spectrometers (nos. 314A, 3375). Samples on filter paper were counted in a solution consisting of 100 mg. of 1,4-bis-(5-phenyloxazol-2-yl)benzene and 4.0 g. of 2,5-diphenyloxazole/l. of toluene. Quenching was corrected for by either the addition of [1,2-³H]n-hexadecane as an internal standard, or the channels-ratio method. The counting solution for aqueous samples was that of Bray (1960). The counting efficiency for ³H was 20-50%, for ¹⁴C 30-80%, depending on quenching.

Materials. ATP, GTP, UTP, CTP, yeast RNA, calf thymus DNA, NADP⁺, 6-phosphogluconic acid and D-glucose 6-phosphate were obtained from Sigma (London) Chemical Co., London S.W.6. Bovine plasma albumin was obtained from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex. Horse heart cytochrome c was from Koch-Light Laboratories Ltd., Colnbrook, Bucks. [8-¹⁴C]ATP (specific radioactivity 14.2 mc/m-mole) and [5-³H]orotic acid (specific radioactivity 5.0 c/m-mole) were obtained from The Radiochemical Centre, Amersham, Bucks. St Martins nylon cloth was obtained from Henry Simon Ltd., Stockport, Cheshire.

EXPLANATION OF PLATE 1

Phase-contrast micrographs of isolated nuclei: (a) $\times 750$; (b) $\times 1800$.

EXPLANATION OF PLATE 2
Electron micrograph of intestinal mucosa ($\times 17250$)

D. E. M. LAWSON, P. W. WILSON, D. C. BARKER AND E. KODICEK

EXPLANATION OF PLATE 3

Electron micrograph of isolated intestinal nuclei ($\times 26000$).

D. E. M. LAWSON, P. W. WILSON, D. C. BARKER AND E. KODICEK

RESULTS

Isolation and morphology of intestinal nuclei of the chick. Plate 1 shows phase-contrast micrographs of the nuclei under low and high magnification. There was no contamination of the nuclei with erythrocytes, and less than one unbroken mucosal cell/500 nuclei. The nuclei appeared as elliptical or occasionally spherical bodies with well defined nucleoli. There were few damaged nuclei present. The average yield of nuclei obtained by method (a) was 15%. The mucosa, debris and isolated nuclei were examined by electron microscopy. In all cases the state of aggregation of the nuclear contents, the integrity of the outer and inner nuclear membranes and the preservation of the mitochondrial and the endoplasmic-reticulum membranes were estimated. The best preservation of the nuclei from the ultrastructural point of view occurred with 1 mM-Ca²⁺ present in all solutions used for the isolation of nuclei. In this way a very close correlation between fine structure in the mucosa and isolated nuclei was observed (Plates 2 and 3). The double nuclear membranes, with ribosomes attached to the outer one, were usually complete, the nucleolus was well differentiated and the nuclear sap appeared as a finely dispersed granular matrix. When fragmentation of the outer nuclear membrane of the isolated nuclei was observed, a similar fragmentation could be seen also in specimens of the debris fraction and of the mucosa, the extent of the damage depending on the molarity and Ca²⁺ concentration of the isolating media. Homogeneity of fractions was estimated by low-magnification electron microscopy of sections taken at different levels throughout the pellet. Higher magnification was used to assess tissue damage.

Aggregation of the nuclear contents occurred in the nuclei isolated by the procedure of Widnell & Tata (1964). Similar aggregation is seen with nuclei isolated by the method of Blobel & Potter (1966) and in procedure (b) adopted in later stages of our investigation. Maggio, Siekevitz & Palade (1963) showed that this aggregation of the nuclear contents occurs when the concentration of bivalent cations in the isolating solution is greater than 1.5 mM.

Although centrifuging the nuclear-debris fraction through 2.4 M-sucrose gave very pure nuclei the yield was poor, about 15%, but this was eventually improved by suspending the intestinal nuclear-debris pellet in 0.32 M-sucrose and adding the 2.4 M-sucrose solution slowly with thorough mixing at each addition. By this means the yield was raised to 45%, which was comparable with that obtained from other tissues. Løvtrup-Rein & McEwen (1966) have reported that improved yields of nuclei can be obtained with the addition of Triton

X-100, but the use of detergents in this manner for investigations involving lipids is open to criticism.

Isolation and morphology of intestinal nuclei of the rat. Isolation of intracellular fractions from rat intestine is known to be difficult owing to the presence of mucus (Busch, Davis & Anderson, 1958). The yield of rat intestinal nuclei sedimenting through the 2.4 M-sucrose in the present series of experiments was extremely low and variable, and the nuclei were contaminated with mucus and whole cells. Attempts were made to overcome this difficulty by changing the homogenizing medium and by the use of mucolytic agents. However, mannitol (Norman & DeLuca, 1964), glycerol (Schneider, 1955) and saline (Miller & Crane, 1961) and the mucolytic agent *N*-acetyl-L-cysteine (Scheffner, 1963) had no effect on the removal of the mucus, and damaged nuclei were obtained.

Properties of chick intestinal nuclei

Chemical analysis. The ratios of RNA and protein to DNA of a typical nuclear preparation from vitamin D-supplemented birds were 1.14 and 28.5 in the homogenate and 0.15 and 3.1 in the nuclei, respectively. They are in general agreement with those reported on other tissues in the literature (see Siebert & Humphrey, 1965). There was no consistent variation in the rRNA content with differences in the vitamin D status of the birds.

Extranuclear enzyme activities. The purity of the isolated nuclei was determined by assaying the following enzymes as an indication of the presence of other intracellular fractions (Hübscher *et al.* 1965): invertase for brush borders, cytochrome oxidase for mitochondria, glucose 6-phosphatase for microsomes and 6-phosphogluconate dehydrogenase for the cell-sap (Table 1).

RNA polymerase activity. RNA polymerase and NAD pyrophosphorylase (EC 2.7.7.1) are the only two enzymes that are generally accepted to be confined to nuclei (Roodyn, 1963; Siebert & Humphrey, 1965). We measured the Mg²⁺-stimulated and Mn²⁺-stimulated RNA polymerase

Table 1. *Proportion of cytoplasmic enzyme markers in isolated nuclei*

The specific activities of the enzymes are expressed as nmoles of substrate converted/min./mg. of protein.

	Specific activity		% of total recovered in nuclei
	Homogenate	Nuclei	
Invertase	156.5	42.7	0.30
Cytochrome oxidase	102.2	13.4	0.16
Glucose 6-phosphate	152	5.3	0.64
6-Phosphogluconate dehydrogenase	17.0	5.4	0.40

Table 2. *Effect of cholecalciferol on the Mg^{2+} -stimulated and Mn^{2+} -stimulated RNA polymerase activities of isolated intestinal nuclei*

Rachitic birds were given cholecalciferol intracardially at various time-intervals before preparation of the intestinal nuclei. The activity of the Mg^{2+} -stimulated enzyme was measured after 15 min. incubation of the nuclei and that of the Mn^{2+} -stimulated enzyme after 45 min. incubation. Results are the average of triplicate determinations, usually at two different enzyme concentrations, calculated in pmoles of total ATP incorporated/mg. of DNA and shown as percentages of the activity of nuclei from rachitic birds.

Amount of cholecalciferol ($\mu g.$)	Time after dosing	Activity (%)	
		Mg^{2+} -stimulated enzyme	Mn^{2+} -stimulated enzyme
2.5	5 hr.	80	—
5	3 weeks	84	—
125	1 hr.	92	87
125	1 hr.	—	84

Fig. 1. Time-course of incorporation of $[8-^{14}C]$ ATP into RNA by the two DNA-dependent RNA polymerases in isolated intestinal nuclei. $\circ$, Mg^{2+} -stimulated RNA polymerase; $\bullet$, Mn^{2+} -stimulated RNA polymerase. The incubation conditions were as described by Widnell & Tata (1964). $[8-^{14}C]$ ATP was added at the times indicated ($\downarrow$).

reactions (Widnell & Tata, 1964; Tata & Widnell, 1966) of intestinal nuclei (Table 2). The omission of the nucleotides CTP, UTP and GTP resulted in 90% loss of enzyme activity. In agreement with these authors, the activity of the Mg^{2+} -stimulated reaction was doubled by the presence of 35 μ moles of KCl and the pH-activity curve was almost flat between pH 8 and 9 with the optimum at 8.6. The activity of the enzyme was proportional to the enzyme concentration with up to 250 $\mu g.$ of DNA in the reaction mixture. The reaction was complete at 15 min. with 220 $\mu g.$ of DNA, and by 30 min. there was a slight fall in the observed incorporation of $[8-^{14}C]$ ATP (Fig. 1). The rate of Mn^{2+} -stimulated RNA polymerase reaction was almost linear up to 60 min. (Fig. 1).

Effect of cholecalciferol on RNA polymerase. The effect of cholecalciferol on the activity of these

two enzymes in isolated intestinal nuclei was measured after exposure of rachitic birds to various amounts of the vitamin for several time-periods. In each case the activity was compared with that from rachitic birds. Some representative results are given in Table 2. With the Mg^{2+} -stimulated enzyme there was in 12 experiments a quite wide variation in enzyme activity from one group of birds to another. There appeared to be a small decrease in enzyme activity after vitamin dosage. For example, there was a 20% inhibition of the enzyme in those rachitic birds given 2.5 $\mu g.$ of cholecalciferol for 5 hr. With the Mn^{2+} -stimulated RNA polymerase there was less variation in enzyme activity, but there was also a small decrease in the activity after treatment with the vitamin.

Effect of cholecalciferol on incorporation of $[5-^3H]$ -orotic acid into chick intestinal RNA.

nRNA. The results of Norman (1966), showing an effect of vitamin D on total tissue RNA 20 min. after administration of the $[^3H]$ orotic acid, together with those on the inhibition by actinomycin D of Ca^{2+} transport (Eisenstein & Passavoy, 1964), suggested that there was an effect of vitamin D on nRNA metabolism. Table 3 shows the increase in the incorporation of $[^3H]$ orotic acid into RNA prepared from isolated nuclei 1 hr. after an intracardial injection of cholecalciferol. The variation in the effect of cholecalciferol is possibly due to lack of uniformity in the vitamin D status of the different groups of chicks.

Tissue RNA after various time-intervals. The effect of cholecalciferol on the incorporation of $[5-^3H]$ orotic acid into nRNA could be obtained as early as 10 min. after exposure to the vitamin when cholecalciferol (125 $\mu g.$) and the $[^3H]$ orotic acid were injected concomitantly (Table 4). The maximal effect was seen 20 min. after injection of the vitamin and the stimulation was still observed after 3 hr.

The total intestinal RNA content, however, was

Table 3. *Effect of cholecalciferol on the incorporation of [5-³H]orotic acid into nRNA from vitamin D-deficient chicks*

In each experiment 125 µg. of cholecalciferol in propylene glycol was injected intracardially into each of three deficient chicks. A further three birds received only propylene glycol. After 40 min. the birds were given an intraperitoneal injection of [5-³H]orotic acid and killed 20 min. later. The RNA was extracted from the isolated nuclei as described in the text. Results are expressed as d.p.m./mg. of RNA.

Expt. no.	Specific radioactivity of RNA	
	Vitamin-deficient birds	Cholecalciferol injected birds
1	4100	7500
2	9900	12000
3	6000	30100

Table 4. *Incorporation of [5-³H]orotic acid into nRNA from intestines of vitamin D-deficient chicks treated with cholecalciferol at different times*

Groups of vitamin D-deficient birds each received 20 µg of [5-³H]orotic acid intracardially 10 min. before being killed. Cholecalciferol (125 µg.) was given intracardially at the various time-intervals indicated before death.

Time after cholecalciferol injection (min.)	Sp. radioactivity of total RNA (d.p.m./mg. of RNA)
*	230
10	410
20	620
30	620
60	640
180	610

* Propylene glycol injected instead of cholecalciferol.

unaffected by the vitamin D status of the birds, being 8.6 and 8.9 mg./g. of tissue in the vitamin D-deficient and supplemented birds respectively.

DISCUSSION

In recent years hyperosmotic sucrose has been used for the isolation of nuclei from animal tissues in a highly purified form (Chauveau, Moulé & Rouiller, 1956; Sporn, Wanko & Dingman, 1962; Maggio *et al.* 1963; Widnell & Tata, 1964; Blobel & Potter, 1966). Such a method is preferable to those using non-aqueous solvents for investigations into the lipid components of nuclei and results in a purer preparation than is obtained with the method using citric acid (Roodyn, 1963). The present results show that the sedimentation of chick intestinal nuclei through 2.4 M-sucrose produces intact enzymically viable nuclei, free of whole cells

and erythrocytes. In addition, the contamination of the nuclei with cytoplasmic components is extremely low. As several authors have discussed (Roodyn, 1963; Siebert & Humphrey, 1965), there is at present no generally accepted criterion for evaluating the purity of a nuclear preparation. The characteristics of these nuclear preparations were such that it was considered that the nuclei were suitable for investigations into a possible effect of vitamin D on nuclear metabolism and into the occurrence of vitamin D or metabolites in the nuclei.

It is generally accepted that the function of vitamin D is at least twofold: the control of Ca²⁺ absorption in the intestine and the deposition of mineral salt in the bones. However, as a result of the time-lag between the administration of the vitamin and the detection of these effects, it is probable that they are the result of a series of biochemical changes initiated by the cholecalciferol. Several effects of vitamin D have been reported in recent years, including alterations in bone citrate content (Dickens, 1941), phospholipid metabolism (Thompson & DeLuca, 1964; Neville & Holdsworth, 1968; Lawson, 1969), Ca²⁺ release by mitochondria (Engstrom & DeLuca, 1964) and adenyl cyclase activity (Neville & Holdsworth, 1969), all of which could conceivably have affected Ca²⁺ metabolism in the intestine and bone. Recently the possibility of a protein carrier for Ca²⁺ has been investigated (Wasserman, Corradino & Taylor, 1968). The question that has to be answered is therefore whether the increased incorporation of nucleic acid precursors into RNA, under the stimulus of vitamin D, is directly involved in the mode of action of the vitamin, or is another of the varied metabolic changes consequent on its initial action.

Since the demonstration by Eisenstein & Passavoy (1964), using actinomycin D, that the stimulation by vitamin D of intestinal Ca²⁺ absorption could only be demonstrated in the presence of protein synthesis, several studies have been made on the effect of vitamin D on RNA synthesis (Norman, 1966; Stohs, Zull & DeLuca, 1967). As a result of these and our investigations it can be shown that there is a rapid increase in the incorporation of [5-³H]orotic acid into nRNA.

It is tempting to draw parallels between the effects on nucleic acid metabolism observed with the hormones, particularly those with a steroid nucleus (Tata, 1968), and with vitamin D. There are, however, several important differences. Thus in the vitamin D-supplemented animal there is no increase in amount of the total intestinal RNA or of nRNA, nor, at least at early periods after a dose of the vitamin, in the total protein content of the intestine. Finally, and perhaps most importantly, the RNA polymerase activity is not increased by

the vitamin. The small decrease in RNA polymerase activity after vitamin D treatment that was observed in these studies may possibly be due to an increase in the nuclear Ca^{2+} content. Because of the inhibitory effect of Ca^{2+} on this enzyme (Weiss, 1960) the nuclei were isolated in a medium containing Mg^{2+} rather than Ca^{2+} . However, some Ca^{2+} probably remains in the isolated nuclei and only a small difference in Ca^{2+} concentration would be required to give the difference in RNA polymerase activity observed by us. In any case no evidence was found for an increase in RNA polymerase activity corresponding to the extent of the increase in the incorporation of orotic acid into RNA. We also could not find an effect by vitamin D on the specific radioactivities of the RNA precursors in the trichloroacetic acid-soluble fraction of the intestine (1654 d.p.m./g. of mucosa/g. body wt., against 1537 d.p.m./g. of mucosa/g. body wt. for the vitamin D-dosed rats). This agrees with the findings of Stohs *et al.* (1967).

Two explanations are possible to account for the effects of vitamin D. First, the vitamin may influence the permeability of the nuclear membrane with a consequent high labelling of the precursor pool. Since this does not appear to occur one would have to postulate a change in only one component of the precursor pools that would not significantly affect the measurement of the total acid-soluble substances.

Secondly, the vitamin may affect the activity of the other factor of the RNA polymerase system that has not so far been investigated, namely chromatin. For example the vitamin D may affect the template capacity of the intestinal chromatin in a manner analogous to that observed for oestradiol and uterine chromatin (Barker & Warren, 1966). In the next paper (Lawson *et al.* 1969) the localization of the major radioactive component of the intestine is shown to be on the chromatin after a dose of labelled cholecalciferol.

REFERENCES

- Barker, K. L. & Warren, J. C. (1966). *Proc. nat. Acad. Sci., Wash.*, **56**, 1298.
- Blobel, G. & Potter, V. R. (1966). *Science*, **154**, 1662.
- Bray, G. A. (1960). *Analyt. Biochem.* **1**, 279.
- Burton, K. (1956). *Biochem. J.* **62**, 315.
- Busch, H., Davis, J. R. & Anderson, D. C. (1958). *Cancer Res.* **18**, 916.
- Carnie, J. A. & Porteous, J. W. (1962). *Biochem. J.* **85**, 450.
- Ceriotti, G. (1955). *J. biol. Chem.* **214**, 59.
- Chauveau, J., Moulé, Y. & Rouiller, C. L. (1956). *Exp. Cell Res.* **11**, 317.
- Dickens, F. (1941). *Biochem. J.* **35**, 1011.
- Eisenstein, R. & Passavoy, M. (1964). *Proc. Soc. exp. Biol., N. Y.*, **117**, 77.
- Engstrom, G. W. & DeLuca, H. F. (1964). *Biochemistry*, **3**, 303.
- Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
- Haussler, M. R. & Norman, A. W. (1967). *Arch. Biochem. Biophys.* **118**, 145.
- Hübscher, G., West, G. R. & Brindley, D. N. (1965). *Biochem. J.* **97**, 629.
- Kodicek, E. (1963). In *Transfer of Calcium and Strontium across Biological Membranes*, p. 185. Ed. by Wasserman, R. H. New York: Academic Press Inc.
- Lawson, D. E. M. (1969). *Nutr. Dieta*, Suppl. (in the Press).
- Lawson, D. E. M., Wilson, P. W. & Kodicek, E. (1969). *Biochem. J.* **115**, 269.
- Løvtrup-Rein, H. & McEwen, B. S. (1966). *J. Cell Biol.* **30**, 405.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Maggio, R., Siekevitz, P. & Palade, G. E. (1963). *J. Cell Biol.* **18**, 267.
- Miller, D. & Crane, R. K. (1961). *Biochim. biophys. Acta*, **52**, 293.
- Neville, E. & Holdsworth, E. S. (1968). *Biochim. biophys. Acta*, **163**, 362.
- Neville, E. & Holdsworth, E. S. (1969). *FEBS Lett.* **2**, 313.
- Norman, A. W. (1966). *Biochem. biophys. Res. Commun.* **23**, 335.
- Norman, A. W. & DeLuca, H. F. (1964). *Arch. Biochem. Biophys.* **107**, 69.
- Porteous, J. W. & Clark, B. (1965). *Biochem. J.* **96**, 159.
- Roodyn, D. B. (1963). *Symp. biochem. Soc.* **23**, 20.
- Scheffner, A. L. (1963). *Ann. N.Y. Acad. Sci.* **106**, 298.
- Schneider, W. C. (1955). *Exp. Cell Res.* **8**, 24.
- Schneider, W. C. (1957). In *Methods in Enzymology*, vol. 3. p. 680. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Siebert, G. & Humphrey, G. B. (1965). *Advanc. Enzymol.* **27**, 239.
- Smith, L. (1955). In *Methods of Biochemical Analysis*, vol. 2, p. 427. Ed. by Glick, D. N. New York: Interscience Publishers Inc.
- Sporn, M. B., Wanko, T. & Dingman, C. W. (1962). *J. Cell Biol.* **15**, 109.
- Stohs, S. J. & DeLuca, H. F. (1967). *Biochemistry*, **6**, 3338.
- Stohs, S. J., Zull, J. E. & DeLuca, H. F. (1967). *Biochemistry*, **6**, 1304.
- Swanson, M. A. (1955). In *Methods in Enzymology*, vol. 2, p. 541. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Tata, J. R. (1968). *Nature, Lond.*, **219**, 331.
- Tata, J. R. & Widnell, C. C. (1966). *Biochem. J.* **98**, 604.
- Thompson, V. W. & DeLuca, H. F. (1964). *J. biol. Chem.* **239**, 984.
- Wasserman, R. H., Corradino, R. A. & Taylor, A. N. (1968). *J. biol. Chem.* **243**, 3978.
- Weiss, S. B. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 1020.
- Widnell, C. C. & Tata, J. R. (1964). *Biochem. J.* **92**, 313.
- Wilson, P. W., Lawson, D. E. M. & Kodicek, E. (1967). *Biochem. J.* **103**, 165.