INVESTIGATION OF CHOLECALCIFEROL-DEPENDENT PROTEIN BIOSYNTHESIS IN THE CYTOSOL OF THE SMALL INTESTINE OF THE RACHITIC CHICK

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1. Introduction

Definite progress with regard to the discovery of the exact nature of the biosynthesis of the cholecalciferol-dependent calcium-binding protein (CaBP) has been made [1,2]. It has been demonstrated that the active metabolite of cholecalciferol, 1,25-dihydroxycholecalciferol gives rise to a specific mRNA which induces synthesis of one or more cellular proteins concerned with the movement of calcium across the cell. This investigation has demonstrated that 3 cholecalciferol-dependent protein components are detectable, one of which is CaBP.

2. Materials and methods

2.1. *Animals*

One day old Rhode Island X Light Sussex chicks were obtained from the National Institute for Research in Dairying, Shinfield, Reading. The chicks were used when they were 4 weeks old having been fed upon the rachitogenic diet described [3].

2.2. *Chemicals*

Crystalline cholecalciferol (activated 7-dehydrocholesterol) was obtained from Peboc Limited, Middlesex. Prior to intracardial injection propylene glycol was added to an ethanolic solution of cholecalciferol such that the ratio of propylene glycol to ethanol was 9:1 (v/v), and 0.1 ml was used. The radioactive amino acid leucine was obtained from the Radiochemical Centre, Amersham as L-[1-^{14}C]leucine and L-[4,5-^{3}H]-leucine and was made up in 0.9% NaCl before intracardial injection of 0.1 ml.

2.3. *Tissue preparation*

These double-isotope-labelling experiments involved the use of [^{14}C]leucine and [^{3}H]leucine in groups of 3 chicks, dosed and undosed, with cholecalciferol consecutively at 4 separate time intervals. Thus 12 chicks fed upon the diet mentioned earlier from 1 day old were injected intracardially at 4 weeks of age with 5 μg cholecalciferol. Three chicks were killed 4, 8, 12 and 24 h after cholecalciferol administration, these chicks having also received 40 μCi [^{14}C]leucine injected intracardially 1.5 h before slaughter. A further 12 rachitic chicks undosed with cholecalciferol were killed simultaneously, 3 at each time-interval having been injected with 60 μCi [^{3}H]leucine 1.5 h before. The first 15 cm of small intestine following the pylorus was excised, washed through with ice-cold 0.9% NaCl, slit longitudinally and blotted. The mucosal tissue was scraped off using a spatula and homogenised in buffer (pH 7.4) (20%, w/v) with a Potter-Elvejhem homogeniser and Teflon pestle. The composition of the buffer used was 0.0127 M Tris, 0.12 M NaCl, 4.74 mM KCl and 1 mM mercaptoethanol adjusted to pH 7.4 with N HCl [3]. The homogenate was spun at 105 000 X g for 1 h in an MSE Superspeed centrifuge to provide the soluble fraction of the cell. These fractions from each group were mixed at the same time interval so that the number of ^{14}C and ^{3}H dpm values were the same.

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2.4. *Polyacrylamide disc gel electrophoresis, gel slicing and radioactive counting*

After mixing the cytosol samples from both cholecalciferol-dosed and undosed groups in the ratio 1:1 (v/v) 500 μl were applied to 15% polyacrylamide gels. Composition of the solutions and the methodology used during electrophoresis are given in [4]. Duplicate gels were run for each time interval, one gel was stained and the other was frozen in dry-ice before being sliced into 1 mm slices using a macrotome GTS-1 (Yeda Ltd., Rehovot). These slices were placed in scintillation vials with 1 ml 90% NCS in water and incubated in an oven at 60°C for 3 h. The vials were cooled and 10 ml scintillation fluid [4] was added for radioactive counting in a Packard Tri-Carb Automatic Liquid Scintillation Counter.

3. Results

Figure 1 indicates the distribution of radioactive amino acid incorporation into chick cytosol protein

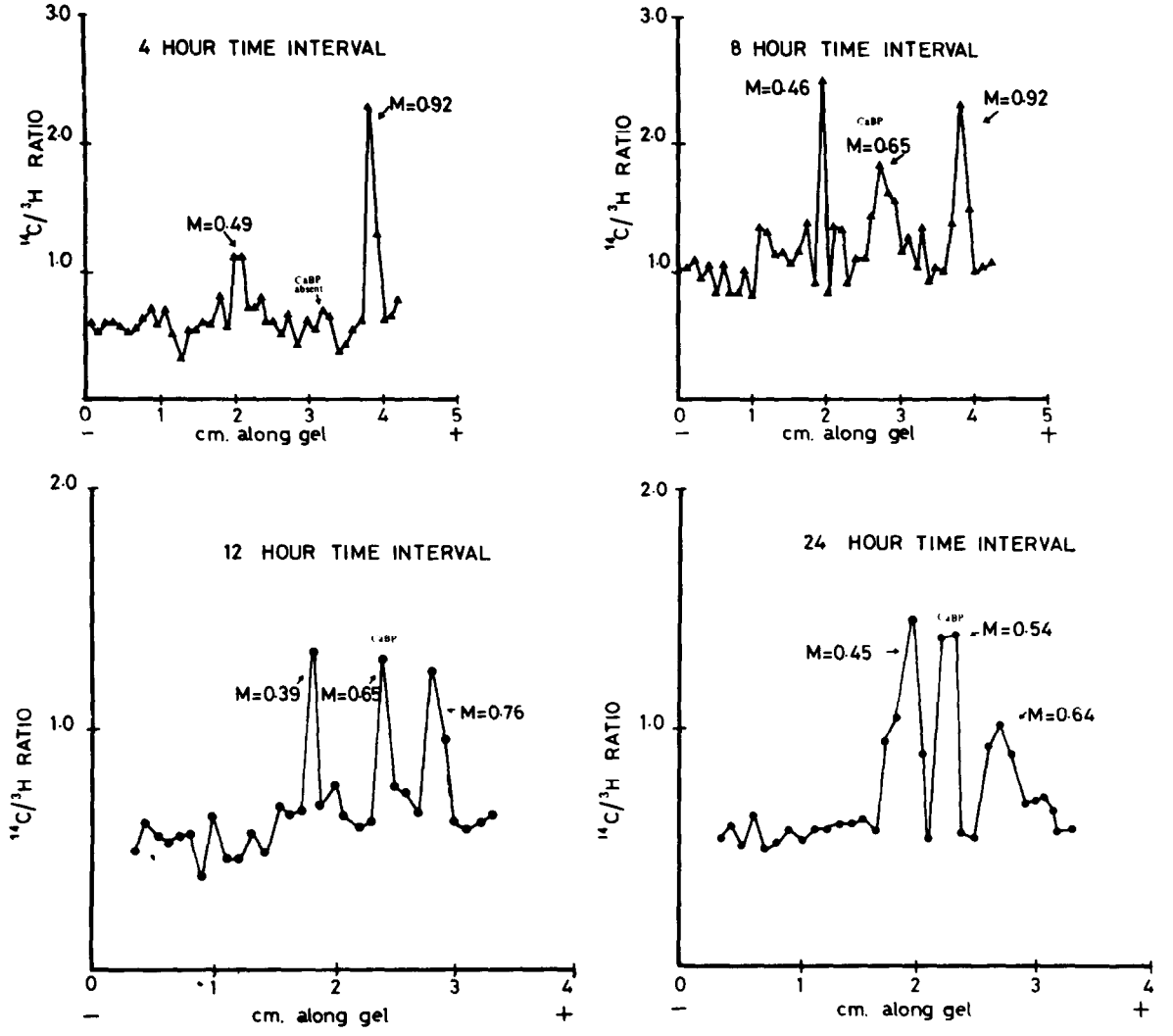

Fig.1. Effect of cholecalciferol on the distribution of labelled leucine incorporation ($^{14}C/^{3}H$) into chick cytosol protein at various intervals using polyacrylamide disc gel electrophoresis.

at the 4 time intervals studied. At the first time interval of 4 h, only 2 radioactive peaks are observed indicating the presence of 2 cholecalciferol-dependent protein components, none of which migrates at the same rate as the prepared sample of chick intestinal CaBP [3] run in parallel. At the remaining 8, 12 and 24 h time intervals 3 cholecalciferol-dependent protein components are detected one of which migrates at the same rate as the cholecalciferol-dependent CaBP.

4. Discussion

The presence of 2 cholecalciferol-dependent protein components 4 h after injection of cholecalciferol could be interpreted as meaning either that they are both association and dissociation products of CaBP or that protein biosynthesis other than CaBP has been detected. The other 3 time intervals following injection with cholecalciferol indicate the presence of 2 components as well as a third component which migrates at the same mobility as CaBP. It is interesting to note that it was observed [5] that following prolonged storage, purified bovine intestinal CaBP gives rise to 2 further protein components, i.e., a total of 3 components. This study has shown the detection of 3 cholecalciferol-dependent protein components present shortly after dosage of the rachitic chick with cholecalciferol. It is a possibility therefore that the 3 components found in this work using polyacrylamide gel electrophoresis are all subunits of CaBP.

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