Serum protein depletion by cultured post-implantation rat embryos[1]

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Summary. Four rat serum proteins were found to be selectively depeleted from the culture medium by 10 day rat embryos. These were α_2-macroglobulin, transferrin and 2 other proteins which had molecular weights of 132,000 and 214,000. Possibly a 5th protein, α_1-acid glycoprotein, was also depleted but its concentration was already low at the start of culture.

Mammalian post-implantation culture techniques have advanced significantly in recent years[3]. These advances have led to studies of the growth factor requirements of developing embryos. Using equilibrium dialysis of serum prior to use as culture medium it was clearly shown that the mcacromolecular fraction was essential to normal development[4,5]. Only one study attempted to analyze the essential components of the macromolecular fraction[6]. The results suggested that a 125,000 and 2 larger than 200,000 mol.wt proteins were utilized by cultured rat embryos. The present report describes an approach to the study of serum macromolecular requirements using gradient-pore polyacrylamide gel electrophoresis (G-PAGE).

Materials and methods. Time-mated Sprague-Dawley rats were used on day 10 (plug day = day 0) and explanted by the method of New[7]. At explantation embryos had between 5 and 12 somites. Rat serum used as the culture medium was prepared by the 'immediate-centrifugation' method and heat-treated at 56 °C/30 min[8,9]. Serum was mixed with Waymouths MB752/1 medium containing 100 IU/ml penicillin and 100 µg/ml streptomycin in the proportion of 7:1 respectively. Explanted embryos were cultured by a roller bottle method[10,11].

Four embryos were usually grown in 4 ml of medium for 48 h except for 1 experiment where the medium was re-cycled in an attempt to intensify the differences between pre- and post-culture medium. In this case 6 embryos were cultured in 1 ml of post-culture medium for 24 h.

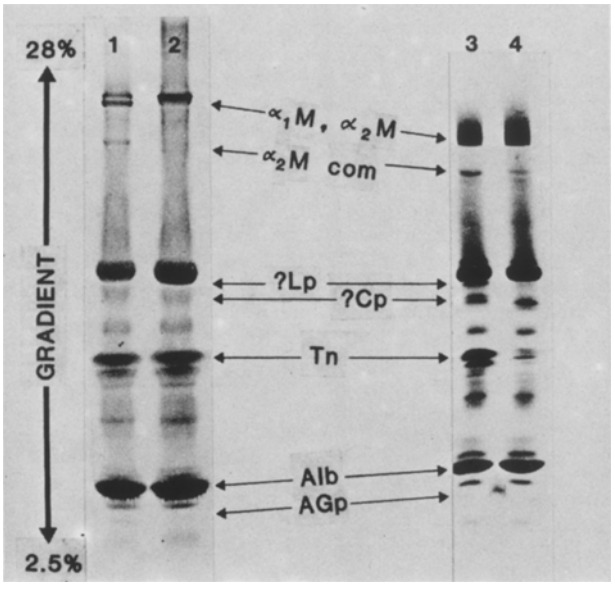

Figure 1. Gradient-pore polyacrylamide gel electrophoresis of rat serum before and after embryo culture. Tracks 1 and 3, pre-culture serum protein profile; track 2, post-culture protein profile; track 4, recycled culture medium post-culture protein profile. Arrows denote migrational position of α_1 macroglobulin (α_1 M), α_2 macroglobulin (α_2 M), α_2 macroglobulin-associated component (α_2 M com), α_1 lipoprotein (? Lp), ceruloplasmin (? Cp), transferrin (Tn), albumin (Alb) and α_1 acid glycoprotein (AGp). Gels 1-2 and 3-4 were photographed at different magnifications.

Serum macromolecules were examined in pre- and post-culture media using a 'Uniscil' Vertical Gel Apparatus (Universal Scientific Ltd, London) with 2.5-28% continuous gradient polyacrylamide gels. A circulating Tris-glycine buffer, pH 8.6 and a current of 68 mA was applied for 40 h. Proteins were stained with Kenacid Blue (BDH Ltd) and glycoproteins with Alcian Blue 8GX[12]. Samples were stored at 4 °C until assay and if more than 4 days delay was unavoidable they were frozen. Freezing did not alter the protein profile. Molecular weights were estimated using a reference collection (Boehringer GmbH, Mannheim) and purified rat α_1- and α_2-macroglobulins[13].

Results. Using G-PAGE a total of about 19 rat serum proteins could be distinguished (fig. 1). Proteins were identified by reference to purified material, published molecular weight and serum concentration data, and their carbohydrate content[14]. When single-use media were compared with unused media held at 4 °C or 37 °C they appeared to have reduced amounts of α_1-acid glycoprotein (Orosomucoid) and α_1- and/or α_2-macroglobulin (fig. 1). However, in some experiments the α_1-acid glycoproteins stained faintly such that no reductions in staining intensity were discernible after culture. The changes in staining intensity did not occur when medium was incubated in the absence of embryos. In the rat, two macroglobulins designated α_1- (α_1M) and α_2- (α_2M) have been described[13,15], but in our system there was a 3rd macroglobulin component running slightly ahead of the other 2 (estimated mol. wt of 380,000). When whole rat serum was co-electrophoresed with purified α_1-M and α_2-M it was shown that the 380,000 protein was a component of α_2-M. The 2 larger protein positions (with mol. wts of about 760,000) were designated 'a' and 'b'; with 'a' exclusive to α_1M and 'b' shared by both α_1- and α_2-M (fig. 2). It thus appeared that α_2-M was the protein being depleted from the medium during culture.

In the re-cycled medium 3 additional proteins were seen to be depleted. These were thought to be transferrin (fig. 1) together with 2 other proteins which had mol.wt of 214,000

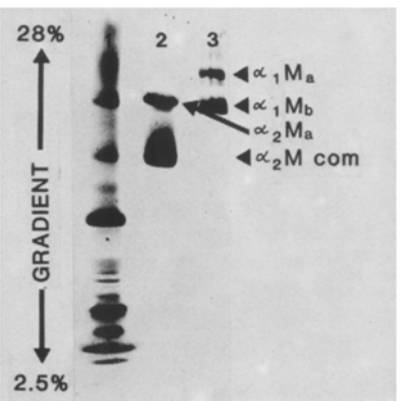

Figure 2. Gradient-pore polyacrylamide gel electrophoresis of rat serum and purified α_1- and α_2-macroglobulins. Track 1, rat serum; track 2, purified α_2-macrogobulin (α_2 M_a and α_2 M com); track 3, purified α_1-macroglobulin (α_1 M_a and α_1 M_b). Samples were electrophoresed for 22 h using a short-form gel.

and 132,000. Although not positively identified, their molecular weights suggested that they might be α_1-lipoprotein and ceruloplasmin respectively. The change in the 132,000 protein appeared to be qualitative and consisted of changes in the migration patterns of 3 apparent forms.

Discussion. The present work demonstrates that certain serum macromolecules are depleted preferentially from the culture medium by developing rat embryos. The uptake of proteins to the visceral yolk sac and embryo was not studied. Recently it has been suggested that albumin is taken up by the visceral yolk sac and digested before passage to the embryo[16].

In the only other report that examined protein utilization by cultured embryos, a 125,000 and 2 greater than 200,000 mol.wt proteins were depleted in the culture medium[6]. It is not clear whether any of these correspond to any of the depleted proteins we detected. It should be noted that our electrophoretic system was non-dissociating and it is likely that the actual molecular weight of the 2 unidentified proteins differ from the apparent molecular weights in our system. However, it supports the contention that certain serum macromolecules are selectively taken up by the conceptus, and may be helpful in defining the protein requirements of developing embryos. Transferrin in a defined medium has recently been shown to support the differentiation of mouse kidney tubules in vitro[17]. Likewise, α_2-M and α_1-acid glycoprotein have been shown to be sufficient to support the growth of primary embryo fibroblasts[18,19]. Whether a concoction of several or all of the above proteins in an artificial medium will be sufficient to support normal whole-embryo development remains to be determined.

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Dietary carotenoids block photocarcinogenic enhancement by benzo (a)pyrene and inhibit its carcinogenesis in the dark[1]

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Summary. The carotenoids β-carotene (C) and canthaxanthine (CX), with and without pro-vitamin A activity, respectively, when perorally administered to mice, markedly prevent benzo(a)pyrene photocarcinogenic enhancement (BP-PCE), continue to block such BP-PCE and protect significantly against BP carcinogenesis in mice maintained in the dark. These results appear relevant to both the pathogenesis of chemical carcinogenesis and rational programs of skin cancer prevention in humans.

In the last decade, experimental trials have suggested that a diet rich in red carrots inhibits the appearance of tumors induced by dimethyl-benz-[a]-anthracene in mice[3] and that injection or peroral administration of carotenoids delays skin tumor induction in hairless mice exposed to UV-B (290–320 nm) irradiation[4,5]. We approached the study of a possible skin cancer modulation by carotenoids, applying our experimental model which proved that non-carcinogenic long UV-irradiation (300–400 nm) strikingly enhances skin benzo(a)pyrene (BP) carcinogenesis in mice[6]. This suggested that in the epidemiology of skin cancer in humans, where chronic sun exposure is recognized to be the major etiologic factor[7], concurrent chemical factors, regardless of their exogenous or endogenous origin, should play an important role. Our experimental trial demonstrated that β-carotene (C) and canthaxanthine (CX), 2 carotenoids, respectively, with and without pro-vitamin A activity, when perorally administered to mice, inhibit and later block BP photocarcinogenic enhancement (PCE)[8]. In the present report the comprehensive results of such study are referred to point out their implication with regard to both the pathogenesis of BP carcinogenesis and possible rational programs of skin cancer prevention in humans.

Materials and methods. C and CX were perorally administered to female Swiss albino mice, strain 955, with daily diet (2.5 mg of C or CX in 5 g of pellets, the latter being the amount of food consumed daily by 1 mouse weighing about 25 g). One month later, the mice were given additional administrations of C and CX, dissolved in arachidic oil, by catheter twice a week (25 mg per 0.250 kg of b.wt).