MECHANISM OF FOETAL WASTAGE FOLLOWING IMMUNONEUTRALISATION OF RIBOFLAVIN CARRIER PROTEIN IN THE PREGNANT RAT

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1. Introduction

In the course of our attempts to understand the phenomenon of facilitated transplacental transport and foetal accumulation of riboflavin in pregnant higher animals and humans [1,2], we found biochemical [3] and immunological [4] evidence for the occurrence of a reproduction-specific, high-affinity riboflavin carrier protein (RCP) in the pregnant rat serum. The rodent vitamin carrier exhibited immunological cross-reactivity with purified chicken RCP, which, in the avian system is obligatory for adequate deposition of the vitamin in the egg yolk for proper development of the prospective embryo [5,6]. The functional importance of the maternal RCP in foetal development and proper progression of pregnancy in the rat was demonstrated by acute foetal wastage and abrupt pregnancy termination resulting from immunoneutralisation of the endogenous RCP with specific and potent antiserum (a/s) to either the avian [4] or rodent vitamin carrier (unpublished). Here, we show that pregnancy termination due to passive immunoneutralisation of RCP is the direct consequence of drastic curtailment of the vitamin supply leading to steep decrease in flavin content and arrest of growth culminating in foetal mortality.

2. Materials and methods

[2-^{14}C]Riboflavin (31 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks). L-[U-^{14}C]Ornithine hydrochloride (250 mCi/mmol) and [^{3}H]progesterone (101 Ci/mmol) were products of New England Nuclear Corp. (Boston MA). The sources of other chemicals and reagents used were the same as in [5,6]. The strain of adult female rats and their maintenance were as in [4]. Their estrous cycles were monitored by microscopic examination of vaginal smears. They were mated during estrous period with adult males of proven fertility and the day on which sperms were detected in the vaginal smears was taken as day 1 of pregnancy. Rabbit a/s to purified chicken RCP was raised and characterised as in [6] and could neutralise 300 μg of the antigen/ml at equivalence point. Both this a/s and the non-immunised rabbit serum (used as control) were incubated for 30 min at 52°C to inactivate the complement, if any, before intraperitoneally administering to rats on day 11 of pregnancy. For measuring embryonic uptake of [^{14}C]riboflavin, the pregnant animals were injected intraperitoneally the labelled vitamin (1 mCi/animal) 1 h after antiserum treatment (0.5 ml/animal) and the embryos were dissected at specified intervals. For quantifying [^{14}C]riboflavin uptake the embryos were homogenised at 4°C in phosphate-buffered saline (0.15 M NaCl in 0.05 M sodium phosphate buffer (pH 7.2)) and an aliquot of the clarified homogenate (spun at 1000 × g, 10 min at 4°C) was digested with hyamine hydroxide and counted for radioactivity by liquid scintillation spectrometry [7]. The total flavin content in embryonic extracts was quantified fluorimetrically after deproteinisation and neutralisation to pH 7.2 [8]. Progesterone levels in the maternal sera were estimated by the specific radioimmunoassay for the steroid [9]. Ornithine decarboxylase activity (ODC) in embryonic extracts was quantified [10] by measuring $^{14}CO_2$ liberated from [U-^{14}C]ornithine. Protein was estimated as in [11].

3. Results and discussion

The time course of foetal uptake of [^{14}C]riboflavin administered to the rats on day 11 of pregnancy and corresponding profiles of fresh weight and ODC activ-

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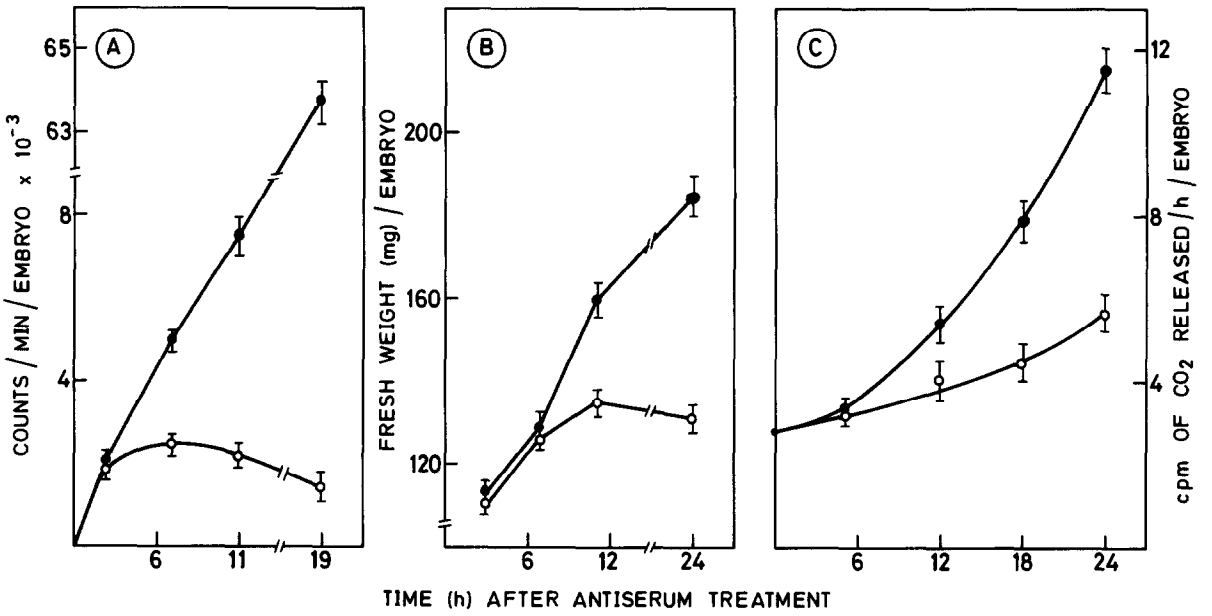

Fig.1. The effect of administration of chicken RCP a/s to pregnant rats on: (A) [^{14}C]riboflavin uptake by embryo; (B) embryonic weight; (C) embryonic ODC activity. The ODC assay reaction mixture (1.0 ml) consisted of 100 μmol Tris–HCl buffer (pH 7.2), 50 nmol pyridoxal phosphate, 2 μmol dithioreitol 50 μg catalase, 1 × 10^5 cpm of L-[U-^{14}C]ornithine and ~200 μg protein from clarified supernatant of embryo homogenate. The incubation was carried out at 37°C for 1 h. Control (•); a/s-treated (○). Vertical bars represent SD (n = 5).

ity of the foeto–placental unit over 24 h are depicted in fig.1. It is clear that in non-immunised control animals, steep linear increase with time of the labelled vitamin influx (fig.1A) was paralleled by a similar gain in fresh weight (fig.1B) and a large and sustained enhancement in ODC activity (fig.1C). The choice of ODC as an additional index of growth is based on recent observation that this first and rate-limiting enzyme of polyamine biosynthetic pathway in mammals [12], progressively increases in the developing rodent embryo registering peak activity on day 12 of gestation (remaining elevated thereafter) and that its irreversible inhibition by D,L-difluoro-ornithine markedly suppresses foetal growth leading to resorption [13]. In sharp contrast to this pattern, in a/s treated animals, there was pronounced curtailment of embryonic uptake of [^{14}C]riboflavin which started manifesting as early as 3 h after a/s dosing and accounted for ~95% inhibition in vitamin influx at 20 h (fig.1A). This was closely followed by marked suppression of ODC activity (perceptible at 6–8 h) and cessation of growth in terms of fresh weight soon thereafter. Thus, it is evident that foetal wastage and consequent pregnancy termination following treatment with RCP a/s [4] are not non-specific but are unequivocally due to interference with the vital functions of maternal RCP as an obligatory vitamin carrier, indispensable to embryonic proliferation. In view of the temporal relationship among suppressed vitamin uptake and the 2 growth parameters, it would appear that deranged foetal development is the consequence of early acute blockade of vitamin delivery to the developing embryo.

To probe further into the intra-embryonic events culminating in foetal wastage, in the a/s-treated animals, gross changes in the profiles of foetal total flavin contents were examined. Fig.2A shows that in control animals, rapid embryonic growth at this stage of pregnancy was attended by a rather slow and marginal enhancement (25%) in total flavin content, which, however, was not commensurate with corresponding growth increase (85%); in fact, on unit fresh weight basis, foetal flavin content registered an initial slow yet significant decrease reaching a plateau level (130 pg/mg tissue) at 10 h. Since rapid foetal growth was still maintained thereafter, it is conceivable that

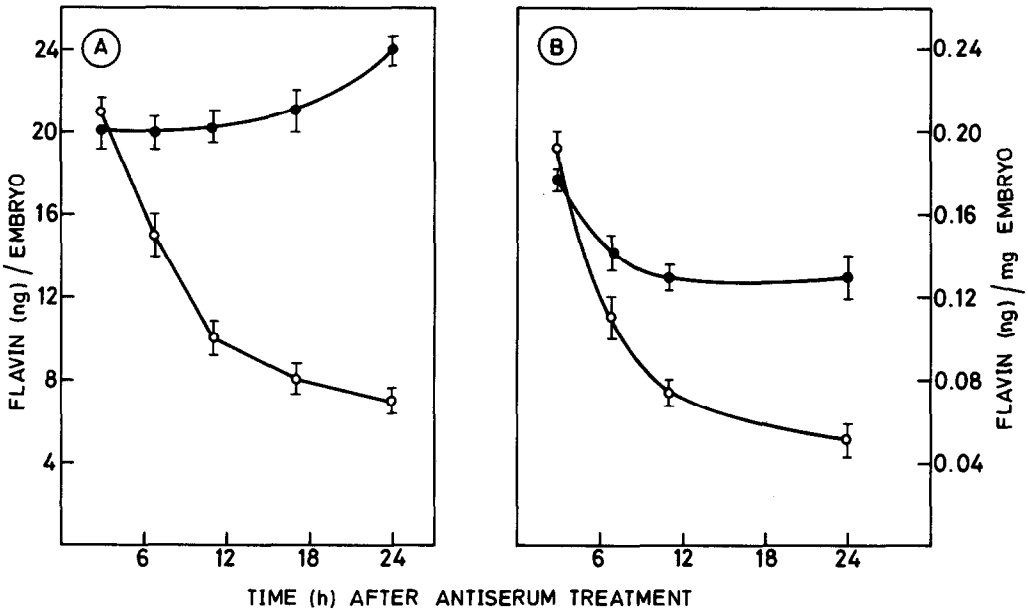

Fig.2. Influence of administration of chicken RCP a/s to pregnant rats on total flavin content of the embryonic tissue. Total flavin content of deproteinised clarified embryonic homogenates was estimated fluorimetrically by measuring fluorescence (reducible by sodium hydrosulphite) emitted at 530 nm upon excitation at 470 nm [8]. Control (•); a/s-treated (○). Vertical bars represent SD (n = 5).

this level of the vitamin represents the minimum threshold value required to sustain continued tissue proliferation. The reasons for these rather unexpected nonparallel increments in flavin contents and fresh weight, despite the continuous vitamin supply, during normal foetal development are not easily explained at present. One distinct possibility that this may be related to fast turnover rate of the vitamin (presumably intrinsic to the embryonic tissue) becomes apparent when the changing pattern of foetal flavin content in a/s-treated animals is examined (fig.2A). Early deprivation of vitamin supply to the foetus resulted in steep and progressive fall in its flavin content (disproportionate to growth inhibition) reaching a value markedly less than that in control animals at a time (6–7 h) when growth retardation was still not discernible. On a unit fresh weight basis, foetal flavin content was clearly less than the plateau level reached in control animals at this period (fig.2B). Thus the accelerated depletion of flavin beyond the critical threshold concentration as a consequence of stoppage of vitamin influx seems to be one of the underlying mechanisms of foetal wastage in the a/s-treated animals.

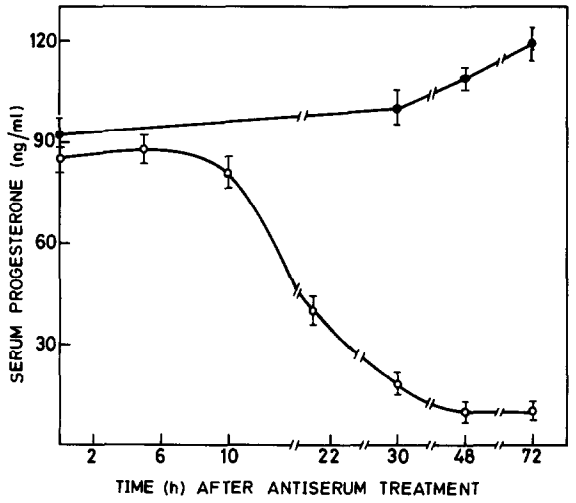

Fig.3. Effects of passive immunoneutralisation of RCP on progesterone levels in 11 day pregnant rats. Sera prepared from blood drawn by cardiac puncture were deproteinised with methanol and steroid fraction was extracted with ether, and the progesterone levels were measured by radioimmunoassay [9]. Control (•); a/s-treated (○). Vertical bars represent SD (n = 5).

Further evidence that these early events lead to foetal degeneration and hence to pregnancy termination was provided by the contrasting pattern of maternal progesterone levels in the a/s-treated animals vis-a-vis in non-immunised animals (controls) (fig.3). Unlike in the controls, characterised by slow but steadily increasing levels of circulatory progesterone [14], in a/s-treated animals, the hormonal concentrations in the maternal serum, precipitously fell from 10 h, signalling foetal distress and consequent shutting off of the luteotropic stimulus from the foeto–placental unit and hence leuteolysis [15].

Thus, apart from unequivocally confirming our observation [4] regarding the paramount functional importance of maternal RCP for continuous supply of riboflavin to the developing foetus in the mammal, these data emphasise that foetal vitamin metabolism is in continuous flux and is highly vulnerable to interference at the level of transplacental transport itself. If is also clear that foetal tissue characterised by fast vitamin turnover rate (which probably accounts for a very low flavin level in the embryo vis-a-vis normal or regenerating rat liver [16]) has limited capacity to survive by neutralising the available intra-embryonic vitamin content. Furthermore, the above data also demonstrate for the first time that immunological interference with the functioning of the carrier protein can be exploited for precipitating selective and acute embryonic vitamin deficiency thus opening new vistas of vitamin research in the foetal tissue.

Acknowledgement

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