with inhibitor-specific antibodies, or for control unspecific rabbit 7S-γ-globulins (Behring Werke AG, Marburg) followed by appropriate washings with phosphate buffered saline. Further controls were done by using inhibitor-specific antibodies incubated prior to the experiments with highly purified HUSI-I. In a 2nd step, goat antiserum directed against rabbit 7S-γ-globulins conjugated with fluoresceinisothiocyanate (Hyland Lab. Inc., USA, Lot No.2232T004A) was employed. For the microscopical examination, a Leitz fluorescence microscope equipped with an automatic camera set (Orthomat, Leitz, Wetzler) was available. Immunodiffusion and immunoelectrophoresis showed monospecificity of the HUSI-I-directed antibodies with formation of a single precipitation line against cervical mucus and cervical homogenate[5,6].

Results and discussion. The results of the immunofluorescent studies are summarized in the figure showing corresponding findings in the 5 investigated female subjects. The antileukoprotease of the human cervical mucus could be localized in the epithelial cell-layer of the upper cervix. In the lower cervix and in the endometrium, no acid-stable proteinase inhibitor was found. This indicates that the antileukoprotease is a specific product of the cervical epithelium. However, immunofluorescence does not allow us to determine whether the identified molecules are biological active or inactive. The probability of active inhibitor molecules is supported from studies showing that the antileukoprotease is a rather stable molecule which is found only in an active form, with no evidence for precursor molecules[4,5,11].

Oestrogen-dependency of the antileukoprotease and changes during the normal menstrual cycle were recently investigated[11]. In contrast to the pattern of different serum proteins in cervical mucus during ovulatory cycles showing lowest concentrations during the periovulatory phase[12], the concentrations of the antileukoprotease remains relatively high around ovulation[11,13]. If the concentration of the acid-stable proteinase inhibitor is referred to albumin, an increased synthesis of the antileukoprotease by the epithelial cells at the time of ovulation can be demonstrated. Present evidence suggests that the cervical mucus proteinase inhibitor is not directly involved in the fertilization process, but is a part of a local defense mechanism of the cervical epithelium with the capacity to neutralize free proteinases.

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2 Requests for reprints should be addressed to: W.B. S., Department of Dermatology, University of Munich, Frauenlobstrasse 9-11, D-8000 Munich 2 (Federal Republic of Germany).

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A synergistic interaction between the teratogenic effect of trypan blue and dietary deficiency in the rat

R.G. Lendon[1]

Department of Anatomy, The University, Oxford Road, Manchester M13 9PT (England), 22 August 1977

Summary. There was an increased incidence, compared to controls, of exencephaly and micropthalmia in the offspring of rats fed a vitamin D deficient diet and injected with trypan blue on day 9 of gestation. Oral vitamin D did not reverse the effect.

Experimental studies have indicated that animals fed vitamin deficient diets or vitamin antagonists during pregnancy can show high incidences of congenital malformations[2]. However few studies have been made on the effect of vitamin D deficiency on pregnancy mainly due to the fact that a suitable diet was difficult to produce. With the recent development of better diets[3] and more accurate serum assays for vitamin D metabolites[4] it seemed reasonable to re-assess the effect of vitamin D deficiency on the outcome of pregnancy, particularly as Davies[5] had suggested that vitamin D precursors might be important for neurulation in the chick. As malformations like neural tube anomalies are probably multifactorial in origin[6] it was also decided to try the effect of injecting a known neural tube teratogen (trypan blue) into animals on the deficient diet.

Materials and methods. The diet used for the experiments was similar to diet II used by Lumb et al.[3] but was not ricketogenic. It consisted of, white flour 4.25 kg; egg albumen 0.5 kg; sodium chloride 0.1 kg; calcium carbonate 0.0475 kg; ferric citrate 0.01 kg; anhydrous disodium hydrogen orthophosphate 0.112 kg. To this was added 0.02 kg of a mixture of B vitamins[7], plus vitamins A (14 mg), E (0.2 g), and K (21 mg). There is no dietary requirement for vitamin C in the rat[8]. The whole mix was made into a dough with water and arachis oil.

5 groups of inbred Wistar-derived rats were used. The 1st group was maintained on normal pellet diet (Oakes PMD) before being timed mated (vaginal plug = day 1). The 2nd group was fed the deficient diet for 3 months from 70 days of age, and were then mated. Group 3 received normal

Numbers of normal and abnormal fetuses (percentage in brackets) after 5 regimes

Group	Number of litters	Fetuses Normal	Resorbed	Exencephaly	Micropthalmia	Total	Litter size (1SD)
1 Normal diet (ND)	25	246 (94.6)	14 (5.4)	-	-	260	10.4 (± 1.96)
2 Deficient diet (DD)	24	214 (96.0)	9 (4.0)	-	-	223	9.3 (± 1.76)
3 ND + trypan blue (TB)	18	110 (59.8)	53 (28.8)	4 (2.2)	17 (9.2)	184	10.2 (± 2.07)
4 DD + TB	19	36 (20.9)	84 (48.8)	20 (11.6)	32 (18.6)	172	9.05 (± 2.09)
5 DD + TB + vitamin D	19	42 (23.1)	89 (48.9)	25 (13.7)	26 (14.3)	182	9.6 (± 1.84)

pellet diet but after timed mating were given a single i.p. injection of 1% aqueous trypan blue (British Drug Houses) on day 9 of pregnancy. The dose was 0.125 g/kg b.wt, a dose found by previous experimentation to produce few neural tube anomalies. The 4th group were fed the deficient diet and mated like group 2 but received a single i.p. injection of 0.125 g/kg of trypan blue on day 9 of gestation. The 5th group received the same treatment as group 4 but in addition were given 100 IU of vitamin D_3 orally each week to keep their serum levels of 25-hydroxycholecalciferol in the normal range.

After 3 months on the deficient diet serum levels of 25-hydroxycholecalciferol ranged from < 2.35–5.6 ng/ml compared with normal values of 15–18 ng/ml. On day 21 or 22 of gestation the rats were killed by chloroform, resorption sites recorded, and live fetuses dissected out and inspected for external malformation. For examination of the skeleton some embryos were cleared and stained with methylene blue or alizarin red. Histology of the limbs was carried out on other fetuses.

Results and discussion. The table shows that no external anomalies were found in either of groups 1 or 2. No skeletal defects were seen after staining with methylene blue or alizarin red, and the histology was also normal. The litter sizes were significantly smaller for animals on the deficient diet (Student's t-test, $p = 0.019$).

The dose of trypan blue that produced 2% exencephaly and 9% micropthalmia in the offspring of rats on a pellet diet (group 3) resulted in a statistically significant increased incidence of these defects (χ^2 with Yates' correction, $p = 0.0012$ for exencephaly, $p = 0.015$ for micropthalmia) when the mothers were on the deficient diet (group 4). The resorption rate was also increased. However, an oral supplement of vitamin D_3 had no protective effect on the incidence of malformation or resorption (group 4), although it did insignificantly increase the litter sizes.

The conclusion to be drawn is that the increased incidence of anomalies relates to some deficiency in the diet other than that of vitamin D. It also stresses the importance of an adequate diet during pregnancy particularly in the presence of known or unknown environmental teratogens. Fasting has previously been shown to be teratogenic in mice[9-11] and to enhance the teratogenic effects of cortisone[9]. In Britain the incidence of anencephaly and spina bifida has tended to be higher in the lower social classes[12] and thus it is interesting to speculate whether an inadequate diet, by enhancing the effect of some unknown environmental teratogen, might be one of the contributory factors in the embryogenesis of neural tube malformations.

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Presence of melanosome-like granules in dermal erythrophores of the tropical teleost *(Badis badis)*

M. Asada[1,2]

Laboratory of Developmental Biology, Department of Zoology, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606 (Japan), 8 September 1977

Summary. Melanosome-like granules were found in dermal erythrophores of the adult tropical teleost, *Badis badis*. Possible mechanisms of their formation are discussed as compared with previously reported examples of the hybrid chromatophore.

Chromatophores of lower vertebrates normally have 1 specific type of pigment organelles, which is characteristic for the cell type. Melanosomes are specific to melanophores, pterinosomes to xanthophores or erythrophores, and reflecting platelets to iridophores. In some cases, however, examples of the hybrid chromatophore[3] have been reported which have pigment organelles of different types in a single cell. At light microscopic level, erythrophores containing melanin granules or both melanin granules and guanine crystals have been reported in integuments of the frog[4]. At electron microscopic level, xanthophores or erythrophores containing melanosomes have been reported in scales of the iguanid lizard[5], integuments of the red-backed salamander[3], and fins of the zebra danio[6]. Iridophores containing melanosomes have been reported in the iris of the Inca dove and the Mexican ground dove[7]. Xanthophores and iridophores containing premelanosome-like inclusions have been reported in integuments of the young