folds. This ionophore-mediated change in cell shape apparently weakened the neuroepithelium as shown by the flattened neural groove and collapsed neural folds (figure 3). This was most apparent in the region where the neuroepithelium was expanding into the midbrain (figure 5). Electron microscopy revealed that the integrity of cytoskeletal components was not visibly altered by ionophore treatment (figure 6). Burnside[17] has suggested that the elongated shape of neuroepithelial cells is not maintained simply by the presence of microtubules, but rather by the directed flow of cytoplasm along the length of these cytoskeletal components toward the cell base. Microfilaments have been shown to be involved in cytoplasmic flow[18,19] and in neuroepithelial cells their coordinated contraction may provide the driving forces for the basally directed cytoplasmic flow. Since the contractile state of microfilaments is regulated by the availability of Ca^{++}[14,20], a possibility exists that ionophore inhibits cell elongation by raising the intracellular concentration of free Ca^{++}, thus disrupting the coordination of microfilament contraction and directed cytoplasmic flow.

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The ontogeny of α-foetoprotein in the chicken

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Summary. The ontogeny of α-foetoprotein (AFP) has been studied in the chicken using polyacrylamide gel gradient electrophoresis and electroimmunoassay from 7 days of incubation until after hatching. The results are discussed in the light of previous on the ontogeny of AFP in mammals.

An embryo-specific serum glycoprotein homologous with mammalian α-foetoprotein (AFP) has been described in the chicken[1]. Studies on the localisation and synthesis of this protein[2] have shown it to be a product of the yolk sac and to a lesser extent the embryonic liver. This is very similar to the situation in mammals, although here the liver appears to be the major site of synthesis[3,4].

The ontogeny of AFP has been studied in man, rodents and rabbits, but relatively little work has yet been done on its avian counterpart. A qualitative study of the changes in the protein composition of embryonic chicken serum (ECS) was made by Weller[5] using immunoelectrophoretic analysis, but no quantitative data on AFP levels during development have been published. This investigation was undertaken to study the ontogeny of AFP in the chick embryo and to compare this with the mammalian situation.

Materials and methods. Pooled 11–15-day embryonic chicken serum was fractionated on Sephadex G200, and the AFP-rich 3rd peak concentrated by pressure filtration. After suitable dialysis this fraction was applied to a column of ConA-Sepharose, the bound glycoproteins eluted with 2% α-D-methylglucoside and concentrated. Final purification was achieved by electrophoresis on a slab on polyacrylamide gel with a monomer concentration gradient of 4–30% (Pharmacia), the pure AFP being eluted from the sliced gel. Sera were analyzed using this same electrophoretic technique.

Electroimmunoassay as originally described by Laurell[6] was used for quantitative AFP determination employing the pure protein preparation as a standard. Total serum protein concentrations were determined by the Lowry technique[7].

Results. Quantitative analyses were not undertaken before 7 days of incubation because of the difficulty in obtaining blood samples free from contamination at earlier stages. However, from 7 days there was a linear rise in total serum protein from just over 10 mg/ml to adult values of around 50 mg/ml 1 week after hatching. During the same period

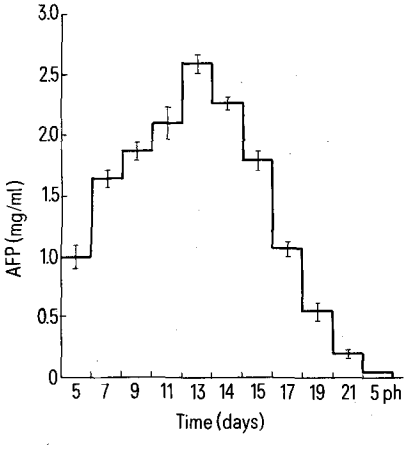

Fig. 1. AFP concentrations in embryonic chicken serum. Values represent the arithmetic mean of 6 individual samples±SE. PH, post hatch.

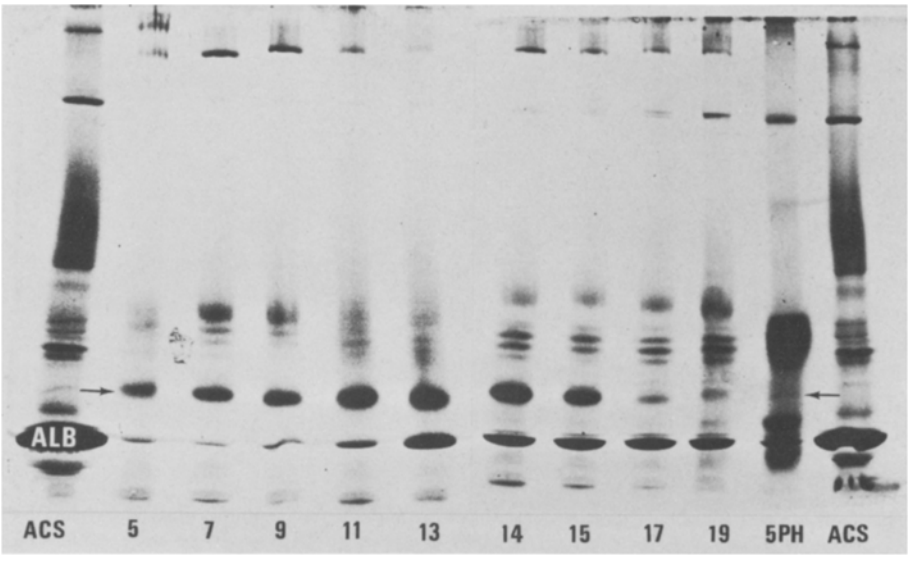

Fig. 2. Gradient PAG electrophoresis of embryonic chick sera illustrating the quantitative changes in AFP throughout development. Arrow, AFP; ACS, adult chicken serum; ALB, albumin; PH, post hatch.

AFP increased from a concentration of 1.65 mg/ml to a peak value of 2.5 mg/ml on day 13, thereafter it declined rapidly to barely detectable levels at 5 days post hatch (figure 1).

As can be seen from the table, at its peak AFP constitutes about 16.5% of the total serum protein, falling to less than 1% at hatching, and effectively disappearing during the first week of life.

These changes in the serum AFP concentration are clearly illustrated by PAG gradient electrophoresis (figure 2). Already prominent at 5 days, the AFP band can be seen to broaden and itensify until day 13, thereafter diminishing quite sharply.

Discussion. A number of similarities can be seen in the ontogeny of AFP between the different mammalian species which have been investigated. The general pattern is a steady build-up during development with maximum concentrations being reached towards the end of the 1st trimestre in humans[8] and rather later in gestation in rats[3] and rabbits[4]. In humans this is followed by an exponential decline in serum AFP, until at term the level has dropped to something like 1–2% of the peak. In rabbits the decline is more gradual, and at birth the AFP concentration is 15–20% of the peak foetal concentration, while in rats only a slight drop is seen as term approaches and levels of 50–60% of the peak are still present at birth.

The situation in the chicken does not compare directly with any of the mammalian ontogenetic patterns, rather it combines features of the rabbit and human. Maximum AFP concentrations are reached fairly late in development as in the rabbit, but the subsequent decline is rapid, as in the human, and at hatching the level is only about 7% of the peak. Peak concentrations of around 2.5 mg/ml are of the same order as has been reported for humans (3 mg/ml), higher levels being found in rabbits (5 mg/ml) and rats (8 mg/ml).

In mammals the yolk sac and the liver are the principal sites of AFP synthesis. The steady increase in AFP levels during early development is due in large part to the increase in size of the liver during this period. In humans the yolk sac, which initially makes a significant contribution to AFP synthesis, undergoes degenerative changes towards the end of the 1st trimestre and the liver subsequently fulfills this function alone. The rapid decline in serum AFP after about 12 weeks gestation is apparently due to a relatively abrupt reduction in the rate of AFP synthesis by the liver[9].

In the rat and the rabbit the yolk sac remains a well developed structure with important biological functions throughout antenatal development and continues to produce AFP until birth. The gradual decline in serum AFP which is seen in the rabbit during the last few days of gestation presumably reflects a combined drop in the rate of synthesis by the yolk sac and liver. At birth the yolk sac is lost and the liver effectively ceases to produce AFP with the result that serum levels continue to fall rapidly during the first weeks of life.

In birds the liver has largely lost the capacity to produce AFP and the well-developed yolk sac is the principal site of synthesis. The somewhat lower peak AFP concentration in the chicken compared with the rat and rabbit might reflect the loss of the liver in birds as a significant source of AFP. The rapid decline in serum AFP levels after 14 days indicates an abrupt reduction in the rate of synthesis by the yolk sac which has effectively stopped by day 19[2].

From these results it would therefore appear that the ontogeny of chicken AFP is broadly similar to that of mammals differing only in detail. The physiological role of AFP during embryogenesis remains unknown and in view of the similarities between avian and mammalian AFP it will be interesting to see whether the immunosuppressive properties which have been recently demonstrated for mammalian AFP[10,11] are shared by avian AFP, and if so what the biological implications of this might be.

AFP as a percentage of total serum protein during chick embryogenesis

Age (days)	Total protein* (mg/ml)	AFP (mg/ml)	%
7	11.2	1.65	14.7
9	12.4	1.88	15.2
11	13.6	2.11	15.5
13	15.8	2.6	16.5
15	18.9	1.81	9.6
17	22.6	1.07	4.7
19	29.0	0.55	1.9
21	32.5	0.212	0.7
5 PH	41.0	0.048	0.1
Adult	49.0	-tive	0

*Determinations made on pooled samples; PH, post hatch.

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Isolation of histone proteins from rat normal and tumour blood plasma

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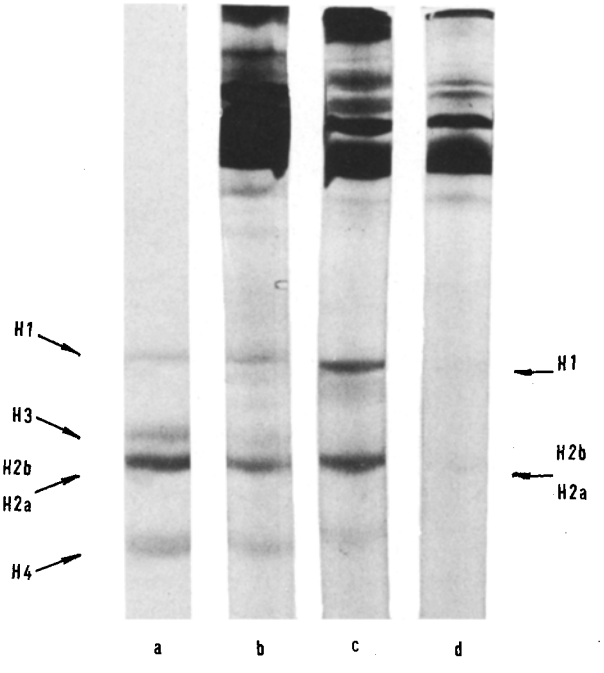
Summary. Histone proteins were isolated from both normal and tumour rat blood plasma: H1, (H2b + H2a) for normal plasma and H1, H3, (H2b + H2a) and H4 for tumour one.

Recently it has been reported that exogenous histones influence cell functions[1,2] and their intracellular structures (lysosimes, mitochondria)[3,4]. Observing the immunological aspects of the inhibitory tumour growth effect of DMS[5], we have established plasma histone-like proteins. The purpose of the present study is isolation and electrophoretic analysis of these histone-like proteins.

Material and methods. Blood plasma from normal Wistar rats and from rats with transplantable 'Joshida' fibrosarcome (diameters of tumour 4–5 cm) was obtained in the usual way by centrifugation at 4 °C. Trichloroacetic acid was then added to a final concentration of 18%. The precipitate was homogenized with 10 vol. of 0.25 M sucrose, 0.1 M tris-HCl pH 7.4 and 0.003 M $CaCl_2$. The homogenate was centrifuged and the sediment was washed twice in the same solution, combining homogenization with centrifugation. The sediment obtained from the last washing was homogenized with 0.25 M H_2SO_4 and was extracted twice more in a similar manner. The combined supernatants were clarified by filtering and histone-like proteins were precipitated by adding 6 vol. ethanol for 18 h at − 10 °C. The precipitate was dissolved in 0.9 N CH_3COOH and 10 M urea. Whole histone was extracted with 0.25 M H_2SO_4 from rat liver chromatin according to Spelsberg and Hnilica[6] and was used as a standard. Acrylamide gel electrophoresis of histones was carried out according to Panyim and Chalkley[7] in 6.25 M urea, 0.9 N acetic acid, pH 3.2. The gels were stained with amido black.



Electrophoretic patterns of histones and cationic plasma proteins. From left to right: a) Histones; b) normal blood plasma + histones; c) tumour blood plasma; d) normal blood plasma.

Results and discussion. Gel electrophoresis of a tumour plasma sample shows 4 cationic protein bands of the same electrophoretic mobility as rat liver chromatin histones: H1, H3, (H2b + H2a) and H4 (figure, c, a). Normal plasma separated into 2 cationic bands corresponding to H1 and (H2b + H2a), which are very faintly visible and difficult to detect compared to the standard histone fractions (figure, d, a). Whole chromatin histone was added to normal plasma immediately before electrophoresis in order to establish if histone mobility is influenced by plasma proteins. In the sample thus obtained, the added whole chromatin histone has the same electrophoretic pattern as that of standard histone and tumour plasma histone (figure, b, a, c). We presume that these blood plasma histone proteins are probably extracted from nucleoproteins present in blood. On the other hand, the former could possibly be histones which have directly entered circulation. In both cases the origin of these histone proteins is most likely related to cell destruction under physiological or pathological conditions. The complicated interaction existing between blood cells and nucleoproteins or histones[1,2] accounts for the difficult isolation of these histone proteins, as well as for the variations in the data we have obtained. What their biological significance is, or if they have only a pathological role in increased cell destruction, is a problem which requires further study.

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