terized by gently applying the fine tip of a tungsten wire loop (actually the filament of a Philips EM-200 electron microscope), minimally heated by a current from a variable, low-voltage a-c source. In 15 animals (including the mouse shown in Fig. 2, h and i) the sinuses were treated electrolytically by inserting a fine needle and passing a current from a variable d-c source (— 6½ volts); the positive lead of the source was attached to the abdomen of the animal. The short circuit adjustment of the power supply (KEPKO ABC-30-0.3M) was set at 50 ma. The two methods of injury seemed to produce identical results.

5. This observation may be explained by insufficient damage to the sense organs and the associated nerve fibers; by (partial) regeneration of nerve fibers and sense organs; or by a certain "refractoriness" of the cortex region involved to damage of the periphery under the conditions of the experiment (for example, the entire barrel field may not have the same "critical period" with regard to its vulnerability to peripheral damage). These alternatives can be experimentally tested.
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Immunoglobulin A: Site and Sequence of Expression in Developing Chicks

Abstract. *Synthesis of immunoglobulins A, G, and M (IgA, IgG, and IgM) is prevented in chickens by embryonic treatment with heterologous antibodies to IgM when combined with bursectomy at hatching. Cells that produce IgA are seeded from the bursa of Fabricius later than cells capable of IgM and IgG synthesis; the latter do not convert to IgA synthesis outside of the bursa.*

Immunoglobulins of the IgM class are synthesized first by developing lymphoid cells of the plasma cell line (B cells) in chickens, guinea pigs, and man (*1*). The following observations suggest that, in chickens, virgin B cells committed to the synthesis of IgG arise within the bursa of Fabricius from cells that formerly made IgM: (i) Removal of the bursa sufficiently early in embryonic life permanently prevents development of all immunoglobulin-producing cells (*2*); (ii) IgG-containing cells are found first in the midst of IgM-containing cells within the bursa and, later, individual cells containing both of these classes can be identified frequently in the bursa, but rarely in the spleen (*1*); (iii) embryonic treatment with heterologous antibodies to IgM (μ chain–specific) suppresses synthesis of IgG as well as of IgM; (iv) IgM-producing cells appear unable to convert to IgG synthesis outside of the bursa (*3*, *4*). At the time of these studies IgM and IgG were the only major classes demonstrated in birds. On the basis of (i) information on the phylogeny and ontogeny of immunoglobulins, (ii) combinations of immunoglobulins seen in cultured human cells, and (iii) patterns of immunoglobulin deficiencies observed in humans, we predicted that cells committed to IgA synthesis also arise from cells that formerly made IgM by a genetic switchover mechanism having the following sequence, IgM→IgG→IgA (*5*). In support of this hypothesis, treatment of mice with antibody to IgM from birth to maturity suppressed synthesis of all immunoglobulin classes (*6*), but the conversion sequence for IgA and IgG heavy chain genes has not been analyzed experimentally. A third immunoglobulin class that is similar to mammalian IgA has been found in chickens (*7*). We now describe studies on the origin of cells committed to synthesis of this class of immunoglobulin and their developmental relationship to B cells committed to IgM or IgG synthesis.

An antiserum specific for chicken IgA was prepared by a modification of the procedure of Lebacq-Verheyden *et al.* (*7*). Pooled bile was dialyzed to remove the salts, filtered over a G-25 Sephadex column, and concentrated. An immune precipitate was then prepared by mixing at equivalence bile and goat antibody to chicken light chains. The washed precipitate, which contained substantial amounts of IgA and only trace amounts of other immunoglobulins was used to immunize goats. The resulting antiserum was rendered specific by repeated passage over immunoadsorbent columns bearing agammaglobulinemic serum, purified IgM, or purified IgG (*1*). When the antibody to IgA was tested by immunoelectrophoresis, a single line was obtained with normal chicken serum and no reaction was detectable with agammaglobulinemic serum, purified IgM, or IgG as antigens. Our antiserum to bile IgA formed a precipitin line with an additional element in bile as described (*7*). Purified antibodies to IgM or IgG, used in our earlier studies (*1*), did not recognize either serum or bile IgA.

The concentration of IgA in the serum of developing chicks was measured by single radial immunodiffusion (*8*). Comparisons were made to pooled serum from normal adult chickens. Minimum amounts of detectable IgA were comparable to a 10 percent dilution of this standard. While lack of a purified serum IgA standard prevented precise quantitation of IgA in serum, dilution studies indicate that the concentration of circulating IgA is far less than the concentrations of IgG and IgM in serum. IgA was not detected

Table 1. Effects of treatment of embryos with heterologous antibodies to IgM or bursectomy at hatching (or both) on serum IgM, IgG, and IgA in chicks at 4 months of age.

Test group	No.	Serum immunoglobulin*		
		IgM* (mg/100 ml)	IgG* (mg/100 ml)	IgA† (%)
Normal chickens	6	124 ± 57	307 ± 108	212 ± 353
Bursectomy at hatching	9	136 ± 66	492 ± 283	36 ± 39‡
Antibody plus bursectomy	17	0	0	0

* Means ± S.D. are given in milligrams per 100 ml and minimum detectable levels were 2 and 5 mg per 100 ml, respectively. † Means ± S.D. are given as percent of a normal adult serum pool, and the detection threshold was 10 percent. ‡ Serum IgA was undetectable in four of nine birds in this group; IgG deficiency (6 mg per 100 ml) occurred in one of nine birds.

in serum from embryos or chicks younger than 19 days of age. By 39 days of age, all of the ten birds in this experiment had detectable amounts of serum IgA (mean, 36 percent of adult standard).

The distribution of IgA-containing cells was examined in blood and in sections of the bursa, spleen, tonsilla caecalis, and nonlymphoid areas of the gut with the IgG fraction of the antibody to IgA labeled with fluorescein isothiocyanate (FITC) (*1*). The distribution of IgA in the bursa is very similar to that of IgM and IgG (*1*); that is, medullary cells of some but not all bursal follicles exhibit a thin rim of fluorescence when stained with FITC-labeled antibody to IgA. Relatively few circulating lymphocytes carry surface IgA [average 0.02 percent for IgA compared to 14.0 percent ± 5.0 (standard deviation) for IgM and 5.4 percent ± 3.1 for IgG]. In keeping with the small amount of IgA in serum relative to that in intestinal secretions (*7*), IgA-containing plasma cells are sparse in the spleen but abundant in the lamina propria of intestinal villi (Fig. 1a), where they constitute a large majority of the immunoglobulin-containing cells. Thus the normal distribution of IgA-producing cells in chickens parallels that defined for mammals (*9*). In addition to plasma cells in the lamina propria, FITC-labeled antibody to IgA stained the brush borders of intestinal epithelial cells and, more intensely, the secretory units of goblet cells (Fig. 1a); this pattern was not seen with antibody to IgM or IgG.

Previous studies with embryonic bursectomy at various times demonstrated that removal of the bursa sufficiently early results in total agammaglobulinemia, whereas slightly later bursectomy often results in animals able to synthesize normal or above normal amounts of IgM but little or no IgG (*2*). We used this approach to obtain information on the relative sensitivity of IgA development to bursectomy. Fifteen chick embryos were bursectomized at 1-day intervals beginning on day 16 of incubation. Under conditions leading to hatching after 20 days of incubation, bursectomy before day 18 of embryonation (nine birds) effectively prevented development of detectable serum IgA by age 5 months or older. Seven of the 15 birds bursectomized as embryos were very deficient in IgG (< 12 mg per 100 ml of serum); all of these lacked detectable amounts of serum IgA. Conversely, deficiency of circulating IgA was observed in bursectomized birds producing large amounts of both IgG and IgM. For example, in two IgA-deficient birds bursectomized at day 17 of incubation, the IgG and IgM concentrations were approximately 1500 and 250 mg per 100 ml of serum, respectively. This suggests that cells irrevocably committed to synthesis of these immunoglobulin classes leave the bursa earlier than cells able to produce IgA.

In order to achieve a more sensitive assessment of IgA-producing capability, these 5-month-old birds were killed, and their tissues were examined by immunofluorescence for cells that contained IgA, IgG, and IgM. No IgA-containing cells were found even in the intestinal lamina propria (Fig. 1b), and no IgA was detectable in bile samples obtained at the same time. Staining of goblet cells in intestinal epithelium was still seen, presumably because in our antibody to IgA (bile source) there were antibodies directed against an element of intestinal secretions other than

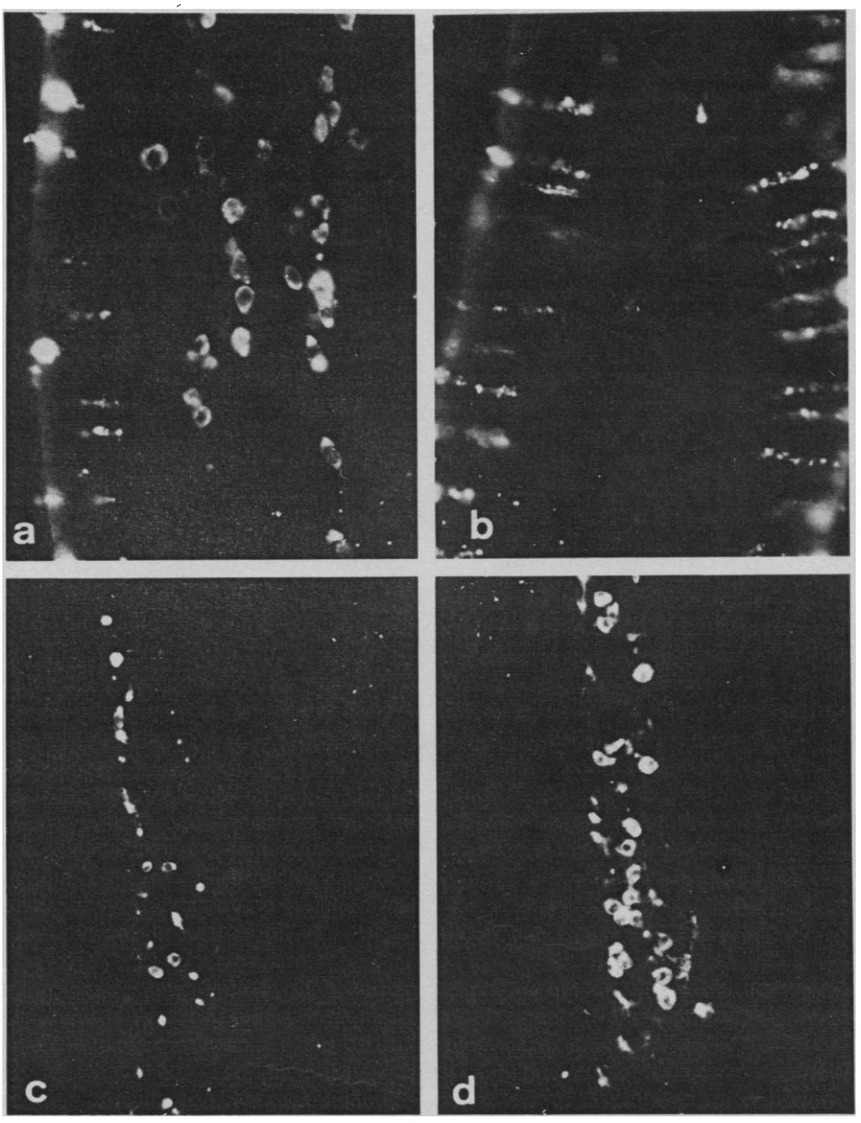

Fig. 1. Intestinal villus sections from 5-month-old normal and embryonically bursectomized chickens; the sections were stained with fluorescein-labeled antibodies to bile IgA and μ chains of serum IgM. (a) IgA-containing cells are numerous in the lamina propria of the normal bird; (b) IgA-containing cells are absent in the IgA-deficient bird, bursectomized at 17 days of embryonic life. Secretory units of the goblet cells and, to a lesser extent, the brush borders of intestinal absorptive cells are stained in both animals; staining in these locations is probably attributable to a secretion-specific antigen associated with IgA in normal bile (7) (a and b, × 265). (c) IgM-containing cells are less numerous in the intestinal lamina propria of the normal bird (× 100); (d) IgM-containing cells are more numerous in the bird with an isolated deficiency of IgA (× 160).

IgA. This assumption is supported by our failure to find goblet cell staining with FITC antibodies to light chains of chicken immunoglobulins. A compensatory increase in the numbers of IgM-containing cells was observed beneath the intestinal epithelium of these IgA-deficient birds (Figs. 1, c and d), as has been seen in humans with selective deficiency of IgA (*10*).

We also examined the effect of antibodies to μ chains on development of IgA-producing cells. Embryonic treatment with antibody to μ chains and bursectomy at hatching, as described (*11*), prevented development of IgA, IgG, and IgM in the serum (Table 1), whereas birds subjected only to bursectomy at hatching made IgM and IgG as well as controls, and most (five of nine) had normal levels of serum IgA. Spleen and intestinal tissues from five of the agammaglobulinemic birds were examined by immunofluorescence, and no cells containing IgA, IgG, IgM, or light chains were found.

These results indicate that cells capable of IgA synthesis (i) arise within the bursa of Fabricius, (ii) follow IgG committed cells in their order of migration elsewhere, and (iii) fail to develop in birds given goat antibodies to IgM heavy chain antigens as embryos and bursectomized at hatching. In several chickens bursectomized as embryos, IgM- and IgG-producing cells did not convert to IgA synthesis. The observations fit our theoretical model (*5*) in which bursal lymphocytes convert over several generations from μ chain synthesis (IgM) through γ chain (IgG) to α chain (IgA) synthesis with random cell migration at all stages of differentiation, but the possibility of direct conversion from expression of μ to α chain genes remains to be excluded. Final solution of this problem could provide information relevant to genetic mechanisms shared by many pathways of cell differentiation.

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Isolation and Structure Determination of Blepharismin, a Conjugation Initiating Gamone in the Ciliate Blepharisma

Abstract. *One of the gamones (gamone II) which are effective for the induction of conjugation in* Blepharisma intermedium *has been isolated in a crystalline form and designated as blepharismin. From the result of chemical and spectroscopic investigations, in which x-ray crystallographic analysis was used as a definitive tool, blepharismin has been found to have the structure of calcium 3-(2′-formylamino-5′-hydroxybenzoyl)lactate.*

Control of conjugation in Protozoa by specific chemical substances has long been assumed (*1*), although their unequivocal identification has been hampered mainly because they are hard to obtain in a soluble form. Recently, the complementary mating types (I and II) of *Blepharisma intermedium* were found to excrete two kinds of gamone (I and II) into the medium, and their characterizations have been reported (*2*, *3*). Of these, gamone II (factor R) (*3*) is responsible for inducing mating type I to conjugate. This report deals with the isolation of gamone II, designated as blepharismin, in a crystalline form and the elucidation of its structure.

Cell-free fluid with gamone II activity (*3*) was concentrated in vacuo to a small volume. The precipitate was removed by filtration. The filtrate was evaporated to dryness and the residue was extracted with absolute ethanol to give partially purified material, which exhibited nine spots under ultraviolet light on the paper chromatogram (Toyo Roshi No. 51; developing solvent, tertiary butanol : concentrated ammonium hydroxide : water, in the proportions 3 : 1 : 1). Of these, only a greenish yellow spot (R_F ratio = 0.56) was responsible for the gamone II activity. Further purification was achieved by column chromatography with cellulose powder (Toyo Roshi 100 to 200 mesh; 75 percent *n*-propanol as eluting solvent) and preparative paper chromatography (Toyo Roshi No. 131). Blepharismin crystallized from water as pale yellow prisms, which exhibited gamone activity at a concentration of 10^{-9} g/ml on mating type I suspended at a density of 500 cells per milliliter. The yield was 3.68 mg from 48 liters of the cell-free fluid with 80 units/ml of gamone II activity.

Blepharismin is a highly polar substance and did not melt below 300°C. The ultramicroanalysis of a partially dried sample (70°C at 1 mm-Hg for 8 hours) showed carbon, 44.70; hydrogen, 4.66; and nitrogen, 4.68 percent. The result of oxygen analysis indicated the presence of some other element, which was determined to be calcium by emission analysis and electron spectroscopy for chemical analysis (ESCA) (observation of $2P_{1/2}$ and $2P_{3/2}$ bands). Undried crystals were estimated to have the molecular weight of 670/2 = 335 (x-ray method) and gave the analytical values of 4.26 and 6.48 percent for nitrogen and calcium (atomic absorption method) respectively. These results, combined with the structural evidence (see below), are best interpreted by assuming that blepharismin has the molecular formula $C_{11}H_{10}NO_6 \cdot 0.5\ Ca \cdot$