*sexualis*, the cross E 87 ♂ (Cambridge, England) × 734 ♀ (Lake Calumet, Chicago) yielded a sample of 56 germinated oospores, which were singly isolated and grown on nutrient media. The mycelia were then mated with each of the parents with the following results: 39 ♂ (25 strong, 12 intermediate, 2 weak in regard to sexual potency); 11 predominantly ♂ (♂ with 734 ♀; induced antheridia on E 87 ♂ and bore aborted oogonial initials in three cases; self-induced antheridia); 6 mixed ♂ ♀ (♂ with 734 ♀; ♀ with E 87 ♂; self-fertile).

In another series of germinations using the same parents, the germ-hypha of one oospore was induced to form a sporangium, from which 19 single zoospore cultures were established and tested. In addition, nine mycelial plugs from various places on colonies produced by each of five different germinated oospores were tested with the parents. In both cases, all products of each single oospore always gave the same result in regard to their sexual interaction and, as far as we could determine, had the same sexual potency.

In matings of *Dictyuchus monosporus* 115 ♂ × 125 ♀ (Cambridge, Mass.), a sample of 36 oospores was germinated and singly isolated on nutrient media. They were then mated with each of the parents with the following results: 18 ♂; 12 ♀; 4 predominantly ♂, and 2 predominantly ♀. The two latter classes yielded, via zoospores, occasional mycelia that mated with neither parent. This apparent sterility, however, probably reflects only the marginal sexual differences between these intermediate isolates and either parent, since failure of interaction between the parental strains themselves is sometimes as high as 10 percent in replicated series of matings. Although we have tested, for several subcultures, large numbers of mycelia derived from single zoospores and mycelial plugs from the germination of individual oospores, we have found no case of male and female isolates derived from a single germinated oospore. We have thus not been able to confirm Couch's (*1*) report of "mixed" strains, which seemed to show some type of vegetative segregation.

Two significant indications may be drawn from these preliminary data on segregation in these forms. (i) The life cycle is probably diploid, the gametes comprising the only haploid phase, although the possibility that meiosis occurs in the germination of the oospore with survival of a single meiotic product cannot now be rigorously discounted. (ii) Control of sexual expression and mating competence resides in a more complex genetic system than paired alleles at a single locus and provide sexual expressions ranging from male through numerous intergradations to female. Of even greater importance, the demonstration of the feasibility of genetic analysis in the biflagellate water molds promises a far more adequate understanding of the biology of this important group of fungi and enhances their already considerable utility for the study of morphogenesis.

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6. A full description of the techniques is being prepared.
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## Immunoglobulin A Production in Ataxia Telangiectasia

Abstract. *Serum from five patients with ataxia telangiectasia contained no detectable immunoglobulin A (IgA). However, there was evidence (by immunofluorescence) of IgA in the bone marrow of the three patients so examined, suggesting that the defect in IgA production was not complete. IgA was in the saliva of all five patients and in the parotid gland of the one patient studied. This is further evidence of IgA synthesis by the salivary glands.*

The immunoglobulins have been divided into the following classes: (i) immunoglobulin A (IgA, $\beta_{2A}$); (ii) immunoglobulin G (IgG, 7*S* γ-globulin); (iii) immunoglobulin M (IgM, 19*S* macroglobulin); and (iv) immunoglobulin D (IgD). These classes are distinguished on the basis of physicochemical and immunological characteristics, and they are present in a variety of body fluids (*1*). Evidence of a salivary and colostral IgA which differs from serum IgA has been presented (*2*).

Serum IgA is absent or markedly decreased in many patients with ataxia telangiectasia (AT), a progressive heredofamilial disease characterized by ataxia, involuntary movements, oculocutaneous telangiectasia, and recurrent pulmonary infections (*3*). Such patients provide an opportunity to study immunoglobulin metabolism, particularly the relation between serum IgA and salivary IgA. We now report on the amounts of immunoglobulin in the serum and saliva of five patients with ataxia telangiectasia. In addition immunofluorescence was used to detect IgA in bone marrow from three patients and in parotid tissue from one patient.

Immunoglobulin concentrations were measured by specific immune precipitation (*4*). Normal values were established from a panel of 50 control serums. The maximum sensitivity of the method was estimated from serial dilutions of standard serums; 0.01 mg of IgA per milliliter could be detected (0.3 percent of mean normal amounts).

The concentrations of IgM in serums were normal and those of IgG varied, but no IgA was detected in the five patients. When the serums were concentrated fivefold, we were still unable to detect IgA (Fig. 1).

Study of immunoglobulin metabolism in these five patients provides evidence that the reduced concentration of IgA in serum is due to decreased IgA synthesis and, in some cases, to an associated hypercatabolism (*5*). To distinguish between severely decreased and absent synthesis of IgA, we have sought additional information by immunofluorescent study of the bone marrow from three patients, two of whom had shown hypercatabolism of IgA. We used a direct immunofluorescent technique (*6*) employing goat antiserum to human IgA, labeled with fluorescein isothiocyanate (*7*). This antiserum showed only antibody reactivity to IgA and did not react with serum from patients with AT (Fig. 2). Just prior to use it was diluted 1:6 and 1:40 and absorbed with guinea-pig-liver powder and serum from patients with AT.

In normal marrow and in marrow from patients with AT, brilliant green cytoplasmic fluorescence was seen in cells of two types; both types were present in approximately equal num-

bers in marrow from normal individuals and patients with AT. Approximately 90 percent of the fluorescent cells were slightly oval with round eccentric nuclei (Fig. 3); these seemed to be plasma cells. The remainder, which were smaller, more round and had a higher nuclear cytoplasmic ratio, seemed to be lymphocytes. This fluorescence could be blocked with unlabeled goat antiserum to IgA but not with rabbit antiserum to IgG or human serum albumin. In order to exclude the possibility of nonspecific fluorescence, the following controls were used. (i) Marrow from a patient with multiple myeloma having an IgG paraprotein was studied by the above method; the myeloma cells were easily identified and were unequivocally devoid of fluorescence; (ii) marrow containing plasma cells (20 percent) obtained from a patient with primary amyloidosis and normal serum immunoglobulins was studied; cytoplasmic fluorescence was seen in only an occasional plasma cell. Thus the antiserum did not react nonspecifically with all plasma cells. This evidence of IgA in bone marrow indicates that the observed serum deficiency in these patients is not due to complete absence of IgA synthesis.

With the immune precipitation technique, IgA was detected in concentrated and unconcentrated saliva from all five patients despite the fact that simultaneous examination of serum failed to reveal IgA (Fig. 4). The amount present in AT saliva was approximately equal to that in saliva from normal subjects.

The identification of salivary IgA led to attempts at cytological localization of its salivary sites in one patient with AT. Parotid tissue was examined by immunofluorescent technique with the goat antiserum to human IgA. Green cytoplasmic fluorescence appeared in scattered acinar cells throughout the tissue (Fig. 5). This was distinctly different from the yellow autofluorescence in untreated parotid tissue and could be blocked with unlabeled goat antiserum to IgA but not with rabbit antiserum to IgG or human serum albumin. Cytoplasmic fluorescence of a few isolated cells (not part of acini) was observed; however, the type of these cells could not be ascertained.

Table 1. Immunoglobulins (mg/ml) in serum of five patients with AT (*8*).

| IgG | IgM | IgA |
|---|---|---|
| | *Patients* | |
| 11.6 | 1.84 | 0* |
| 9.3 | 1.96 | 0* |
| 5.18 | 1.63 | 0* |
| 25.3 | 2.9 | 0* |
| 23.6 | 3.3 | 0* |
| | *Normal (average)* | |
| 12.06 ± 2.65 | 1.45 ± .63 | 2.6 ± 1.07 |

* Less than 0.01 mg/ml.

These findings have bearing on the important question of the origin of immunoglobulins in external secretions. Considerable evidence has been reported previously (*2*) suggesting that IgA can be synthesized in salivary sites: (i) salivary and serum IgA, though immunologically related, were distinguished on physicochemical and immunologic grounds; (ii) immunofluorescent studies gave evidence of IgA presence in normal salivary acinar tissue; (iii) there was no transport of labeled IgA, given intravenously, into saliva. The observation that IgA may occur in saliva of patients with AT further supports the concept that salivary tissue is capable of IgA synthesis. The IgA in saliva of these patients must arise in salivary tissue, unless one assumes that undetectable amounts of IgA were present in serum and that IgA was selectively transported from the serum into saliva. If this were the case, however, after an intravenous injection of IgA labeled with radioiodine, one would expect that protein-bound radioactivity in saliva would have a specific activity equivalent to that of a corresponding serum sample.

An iodinated IgA preparation was available (*5*) and was given intravenously to three patients with AT. Saliva was subsequently collected and showed no protein-bound (nondialyzable) radioactivity in one case and less than 5 percent in two others. Furthermore, the specific activity (counts per minute per milligram of IgA) of IgA in the saliva, if nondialyzable radioactivity

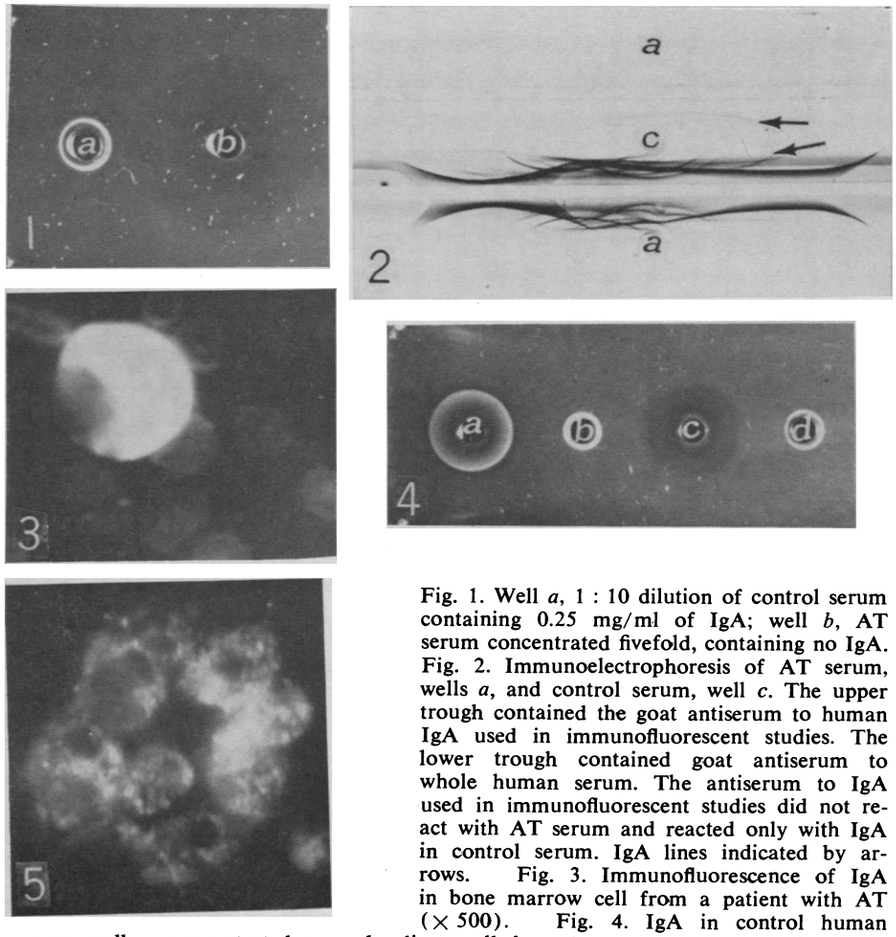


Fig. 1. Well *a*, 1 : 10 dilution of control serum containing 0.25 mg/ml of IgA; well *b*, AT serum concentrated fivefold, containing no IgA. Fig. 2. Immunoelectrophoresis of AT serum, wells *a*, and control serum, well *c*. The upper trough contained the goat antiserum to human IgA used in immunofluorescent studies. The lower trough contained goat antiserum to whole human serum. The antiserum to IgA used in immunofluorescent studies did not react with AT serum and reacted only with IgA in control serum. IgA lines indicated by arrows. Fig. 3. Immunofluorescence of IgA in bone marrow cell from a patient with AT (× 500). Fig. 4. IgA in control human serum, well *a*; concentrated control saliva, well *b*; and concentrated AT saliva, well *d*. No IgA was present in AT serum, well *c*. The diameter of the precipitation ring was related to the concentration of IgA. Fig. 5. Immunofluorescent study of parotid gland of AT patient, showing IgA in acinar cells (× 500).

represents the radioactive globulin, was less than 0.2 percent of the corresponding specific activity in the serum; for this calculation it was assumed that 0.01 mg of IgA per milliliter was present in the serum. This concentration (0.01 mg/ml) of IgA was the maximum amount of IgA which could be present and not detected by the techniques used; if a lower serum concentration of IgA were present, the discrepancy between specific activity of IgA in serum and that in saliva would be even greater. Thus the IgA in the saliva of these patients cannot originate solely from transport from the serum and must arise from another source, presumably in a salivary tissue.

The deficiency of IgA in the serum of the patients studied can be explained by a severe defect of IgA synthesis. However, the immunofluorescent evidence of IgA in the bone marrow suggests that the synthetic defect is incomplete. Other mechanisms such as impaired IgA release or increased catabolism may contribute to the deficiency in serum. Whatever the factors leading to this serum abnormality, there was no apparent defect of IgA production in salivary gland.

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8. These patients have NIH numbers 12715, 12714, 23530, 60424, and 60639.
9. We thank Dr. W. K. Engel for the privilege of studying his patients and for advice during this study.

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## Immunogenicity and Role of Size: Response of Guinea Pigs to Oligotyrosine and Tyrosine Derivatives

Abstract. *Guinea pigs injected with 100 micrograms of* p-*azobenzenearsonate derivatives of hexa-*L-*tyrosine, tri-*L-*tyrosine, or* N-*acetyl-*L-*tyrosine amide, in complete Freund's adjuvant, developed, after 10 to 19 days, delayed-type hypersensitivity to these substances. This was shown by skin reactions, followed by the formation of circulating antibodies that were detectable by passive cutaneous anaphylaxis. Experiments with* p-*azobenzenearsonate–hexa-*L-*tyrosine labeled with iodine-131 showed that this substance was bound in vitro to proteins of normal guinea pig serum. Binding was similar with the nonantigenic hexa-*L-*tyrosine and its* p-*azobenzoate derivative.*

Synthetic polypeptides composed of only one type of amino acid are generally nonimmunogenic (*1*). On the other hand, chemical combination of organic compounds of low molecular weight with homopolymers like polylysine (*2*) or polytyrosine (*3*) may result in antigenic materials.

In a study of the role of the extent of coupling and of the degree of polymerization of various conjugates of poly-L-tyrosine with regard to their immunogenicity in guinea pigs, we found that lightly coupled *p*-azobenzenearsonate–polytyrosine was antigenic even when the polymer had an average degree of polymerization as low as 6, while similar coupling with diazonium derivatives of *p*-aminobenzoic acid and *p*-sulfanilic acid resulted in nonimmunogenic materials (*4*).

We have now examined closely the immunological properties of *p*-azobenzenearsonate derivatives of hexa-L-tyrosine (*5*), tri-L-tyrosine, and *N*-acetyl-L-tyrosine amide (*6*). The lightly coupled (*4*), colored products were purified by dialysis in preheated cellophane tubing (*7*) and lyophilized; *p*-azobenzoate and *p*-azobenzenesulfonate derivatives were similarly prepared and purified. Agar-gel electrophoresis showed that the products were homogeneous in 0.025*M* barbital buffer, *p*H 8.2; each derivative moved toward the anode as a discrete spot. In both hexa- and trityrosine derivatives, an average of one tyrosine residue per molecule was found to be coupled with the haptenic group, as determined colorimetrically (*8*).

Guinea pigs injected in the footpads with single 100-$\mu$g doses of *p*-azobenzenearsonate derivatives of hexa-L-tyrosine, tri-L-tyrosine, or *N*-acetyl-L-tyrosine amide, in complete Freund's adjuvant, developed delayed hypersensitivity to these substances after 10 to 18 days; this was shown by skin reactions, followed by formation of circulating antibodies that were detectable in the serum by passive cutaneous anaphylaxis (Table 1). In all cases studied, the presence of complete Freund's adjuvant in the sensitizing injection was found necessary to effect a positive response. Similar antigenic properties of a *p*-azobenzenearsonate derivative of L-tyrosine were observed by Leskowitz (*9*).

The smallest synthetic polypeptide antigen previously reported was a linear copolymer of tyrosine, glutamic acid, and alanine with a molecular weight of 4000 (*10*). Fibrinopeptide B (molecular weight, 1400) produced antibodies when adsorbed on acrylic plastic particles (*11*). Derivatives of

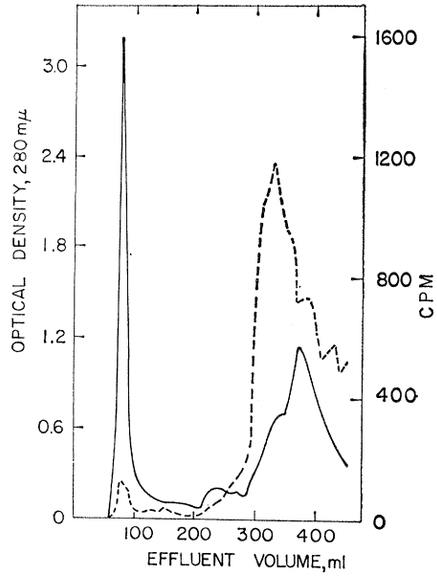


Fig. 1 Elution of normal guinea pig serum (5 ml) mixed with *p*-azobenzenearsonate–hexatyrosine (10 mg) labeled with iodine-131. A linear concentration gradient of 0.02 to 0.3*M* phosphate buffer, *p*H 8, was used on a column of diethylaminoethyl-cellulose. Solid line, ultraviolet absorbancy; broken line, radioactivity; *CPM*, count per minute.