steroids for half-maximum inhibition (I_{50}) of the enzyme are: HA and HDA, $1.3 \times 10^{-7}M$; hellebrigenin (HEL), $3.6 \times 10^{-7}M$; ouabain, $1.4 \times 10^{-6}M$; strophanthidin (STR), $10^{-5}M$. It is of interest to compare the affinities of hellebrigenin and strophanthidin, since their structures differ only in their lactone rings; the affinity of hellebrigenin was 25 to 30 times that of strophanthidin. Our results are similar to those of Glynn (*9*), who found that the bufadienolide, scillaren A, was seven times more potent as an inhibitor of the active transport of K in erythrocytes than the potent cardenolide, digoxin.

When the cardiotonic activities of HA and strophanthidin acetate on the guinea pig atrium (*7*) were compared, very little difference was found. This is an interesting departure from the close correlation between cardiotonic activity and Na-K ATPase inhibitory activity previously found among the derivatives of strophanthidin (*7*) and supports the view, advanced earlier (*2*), that the cardiotonic effect is not brought about by inhibition of the enzyme. It also indicates that variations in the structure of the lactone ring have less effect on cardiotonic activity than on Na-K ATPase inhibitory activity.

In view of the high affinity of the hellebrigenin derivatives for the Na-K ATPase, we prepared hellebrigenin 3-haloacetates in order to study their irreversible inhibition of the enzyme. Hellebrigenin 3-bromoacetate (HBA) was prepared by treatment of a solution of hellebrigenin (300 mg) in dioxane (25 ml, distilled from lithium aluminum hydride) with bromoacetyl chloride (700 mg) at room temperature for 16 hours. The solution was diluted with water (125 ml) and filtered, and the precipitate was dried and crystallized from methanol to yield HBA: 272 mg; $C_{26}H_{33}BrO_7 \cdot H_2O$; melting point, 180° to 182°C; $[\alpha]_D^{25}$, + 26 deg (chloroform solution). The absorption maximum in methanol solution at 298 nm showed a molar extinction coefficient (ϵ) of 5800. (Additional product was obtained by extraction of the filtrate with chloroform and chromatography of the extracted product on silicic acid.) Hellebrigenin 3-iodoacetate (HIA) was prepared in an analogous manner from hellebrigenin (300 mg) and iodoacetyl chloride (750 mg). The dried, pale yellow precipitate (320 mg) was crystallized from methanol, with prior charcoal treatment, to yield colorless prisms: $C_{26}H_{33}IO_7$; melting point, 144° to 145°C; $[\alpha]_D^{26}$, + 26 deg (chloroform solution). The absorption maximum in methanol at 298 nm showed a molar extinction coefficient of 5000 (*10*).

The concentrations of HIA, HBA, and SBA for half-maximum irreversible inhibition of the Na-K ATPase in NaI-treated beef-brain microsomes (*11*) were, respectively, $5 \times 10^{-6}M$, $10^{-4}M$, and $6.6 \times 10^{-4}M$ (Fig. 3). Thus, HIA is about 100 times more potent as an irreversible inhibitor of the enzyme than SBA is and 20 times more potent than HBA. The considerably greater potency of HIA as compared to HBA is consonant with the view that the alkylation reaction is the rate-limiting step and that the superior leaving ability of iodine as compared to bromine plays an important role. It should be noted that the microsomes were washed seven times to remove any unreacted steroid haloacetates. Six washings were required to fully restore the enzyme after incubation with the reversible inhibitor, HA. For very short incubation periods (up to 20 minutes), concentrations of HIA between $10^{-7}M$ and $10^{-6}M$ produced very little irreversible inhibition of the enzyme. It was thus possible to determine the reversible I_{50} for HIA by assaying for short periods in the presence of the steroid, and this was found to be $4.2 \times 10^{-7}M$. This value agrees well with the I_{50} for HA and HDA and indicates that the halogen does not appreciably influence the affinity of the steroid for the site on the enzyme.

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10. Hellebrigenin, HBA, and HIA showed significant cytotoxicity (ED_{50}) against cell cultures of human carcinoma of the nasopharynx (KB) at 10^{-3}, 10^{-6}, and 10^{-6} μg/ml, respectively [see H. Eagle and G. E. Foley, *Amer. J. Med.* **21**, 739 (1956); *Cancer Res.* **18**, 1017 (1958)]. The compounds failed to show significant inhibitory activity against Walker carcinosarcoma 256 in rats at doses up to 100, 25, and 50 mg per kilogram of body weight, respectively. Assays were run, under the auspices of the Cancer Chemotherapy National Service Center, NCI, by the procedures described in *Cancer Chemotherap. Rep. 25* (1962), p. 1.
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Two-Chain Immunoglobulin A Molecules: Abnormal or Normal Intermediates in Synthesis

Abstract. *Immunoglobulin A (γA) myeloma proteins secreted by plasma-cell tumors of mice are of two types, a common four-chain molecule and a rare two-chain (3.9S) molecule. The close similarity between two-chain γA molecules and four-chain γA molecules and their polymers is demonstrated in tryptic peptide maps of isolated polypeptide chains and by precipitin reactions with rabbit antiserums to γA immunoglobulins. However, a difference between these two types is distinguishable with homologous antiserums. Homologous antiserums to two-chain γA immunoglobulins are specific and do not cross-react with four-chain γA immunoglobulins.*

Plasma-cell tumors are readily induced in the inbred BALB/c strain of mice by Lucite shavings, by Freund's adjuvant, and by mineral oil (*1*). Over 50 percent of these tumors synthesize and secrete γA immunoglobulins (*2*). Most γA myeloma proteins are polydisperse in ultracentrifugal analysis, manifesting characteristic peaks with sedimentation coefficients of 7, 9, 11, and 13*S*, and are presumed to consist of four-chain (7*S*) units and polymers (n = 2, 3, and 4) thereof (*3*).

Immunoglobulin production in plasma-cell tumors is often disorderly, and abnormal forms of immunoglobulin molecules are frequently present. The most common types of abnormality observed are either the production of an excess of light chains concomitant with production of the usual four-chain immunoglobulins or the absence of any synthesis of heavy chains. Another type of disorder, so far reported only in mice, is the production

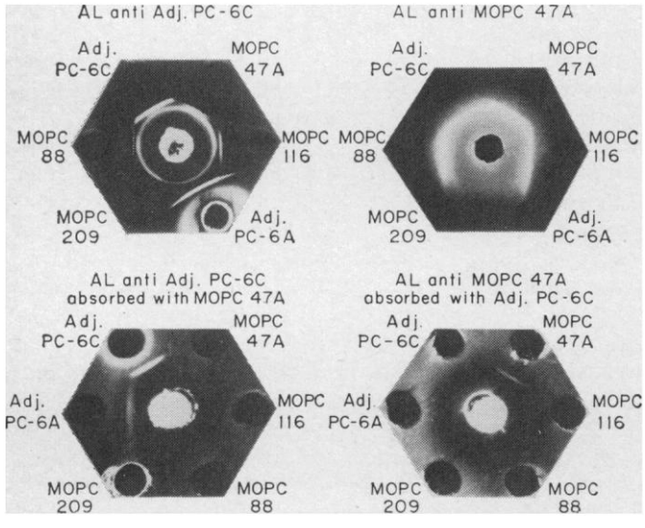
of low-molecular-weight (3.9*S*) urinary globulins which type as γA immunoglobulins with specific rabbit antiserums. Tryptic-peptide maps of whole γA myeloma proteins isolated from urine show tryptic peptides of light- and heavy-chain origin. The total number of tryptic peptides is very similar to that recovered after trypsin digestion of the four-chain γA myeloma globulins (*4*). Investigations (*5*) of the molecular weight of the 3.9*S* γA immunoglobulin and identification of both light and heavy chains suggest that this molecule is composed of a single light and a single heavy chain.

To further characterize this type of molecule, we prepared homologous antiserums which identify a determinant common to all the 3.9*S* myeloma proteins tested.

Of about 300 murine plasma-cell tumors examined, six (Adj. PC-6C, MOPC 4G, MOPC 47A, MOPC 88, MOPC 116, and MOPC 287) produce these two-chain molecules. These proteins are obtained from pooled urine from mice with the above-mentioned plasma-cell tumors. The myeloma proteins were separated from normal proteins in the mouse urine by DEAE-cellulose chromatography (*6*).

Homologous precipitating antiserums were produced in inbred AL and NH mice immunized with 3.9*S* protein from Adj. PC-6C and in AL mice immunized with 3.9*S* protein from MOPC 47A (*7*). Immunization of strains C57BL/6, DBA/2, and BALB/c did not produce any precipitating antibody. All six two-chain γA's were precipitated by each of the three antiserums to the two-chain γA's. Precipitation in Ouchterlony plates of four of the six two-chain γA's with AL antiserum to Adj. PC-6C protein and antiserum to MOPC 47A protein is shown in Fig. 1 (top). Similar results were obtained with NH antiserum to Adj. PC-6C protein (not shown). None of the antiserums to the two-chain γA's precipitated either with normal BALB/c serum, with the serums of 37 other inbred strains of mice, or with other classes of immunoglobulins from BALB/c mice including γM, γF, γG, and γH myeloma proteins and 7, 9, 11, and 13*S* γA proteins. Thus, these two antiserums identified a common "class" specificity in all of the six two-chain γA's, whereas AL antiserum to Adj. PC-6C precipitated the related Adj. PC-6A four-chain γA protein in addition to all the two-chain samples (Fig. 1, upper left). In that these two transplant lines were isolated from the same host, they probably were derived from a common precursor cell. Thus, a specificity common to a two-chain γA (from Adj. PC-6C) and its related four-chain γA (from Adj. PC-6A) was also identified. A third type of specificity, namely, a "tumor specific" or "idiotypic" specificity unique to the immunogen employed, could also be identified. Examples are shown of absorption of AL antiserum to Adj. PC-6C two-chain γA with MOPC 47A protein and of AL antiserum to MOPC 47A two-chain γA absorbed with Adj. PC-6C protein (Fig. 1, lower right and left) where only idiotypic specificities continue to be present.

Since previous studies showed that



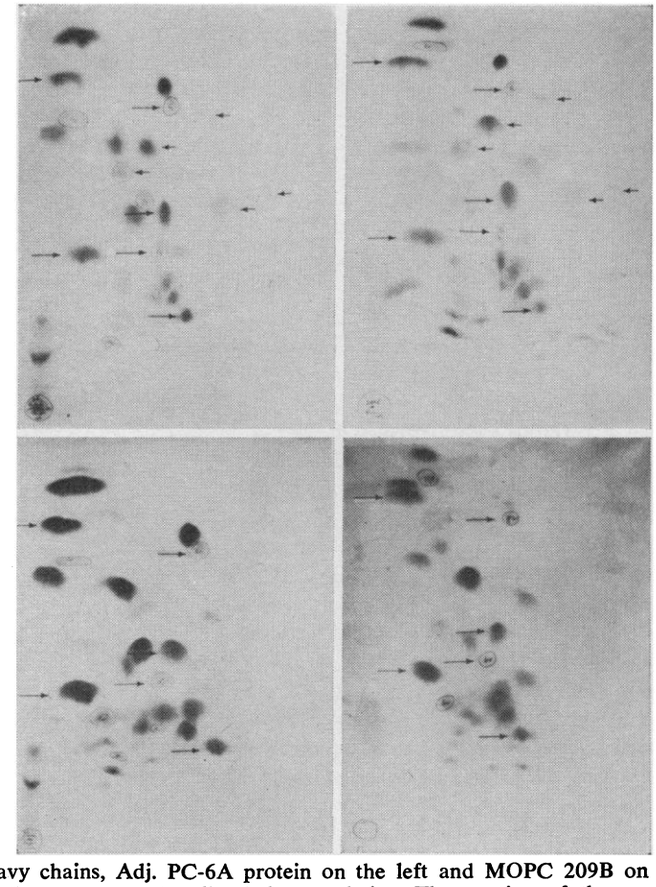


Fig. 1 (above). Upper two patterns: precipitin lines formed between the two unabsorbed antibodies to two-chain γA's (center well) and four two-chain γA's, from Adj. PC-6C, MOPC 47A, MOPC 88, and MOPC 116. No cross-reactions were observed with four-chain γA myeloma proteins from MOPC 209B or Adj. PC-6A, except when antibody was made against Adj. PC-6C protein, which is very closely related to the protein from Adj. PC-6A. Lower two patterns: class-specific antibody to two-chain γA removed from each antiserum by absorption with a two-chain γA different from the one used as antigen in preparing that particular antiserum; only the idiotypic or tumor-specific precipitin lines remain. Fig. 2 (right). Tryptic peptide maps of γA heavy chains; chromatography from left to right and electrophoresis from bottom to top with origin at lower-left corner. Upper two figures are maps of four-chain γA heavy chains, Adj. PC-6A protein on the left and MOPC 209B on the right. The arrows pointing to the right indicate 6 of the 20 peptides common to all γA heavy chains. The spacing of the arrows permits identification of comparable peptides in the four maps. The arrows pointing left mark those five peptides present only in the heavy chains of four-chain γA's.

the papain Fc-fragment contained the class-specific antigenic sites for γA (four-chain), γG, and γH immunoglobulins (*8*), we attempted to localize the two-chain γA class-specific determinant on a papain fragment. However, two-chain γA proteins from Adj. PC-6C and MOPC 47A have not yielded to papain hydrolysis in our laboratory. Thus, lacking Fc- and Fab-fragments from the two-chain γA proteins, we were unable to localize the site of their class specificity.

The heavy chains from the two-chain γA's and those from the four-chain γA's were compared by reduction, alkylation, separation of light and heavy chains, and tryptic peptide mapping (*6*). The maps of heavy chains isolated from two four-chain γA molecules (from Adj. PC-6A and MOPC 209B) and from two two-chain γA molecules (from Adj. PC-6C and MOPC 47A) are shown in Fig. 2. There are about 20 ninhydrin-positive peptides common to all four chains and all other mouse γA heavy chains studied in this laboratory. The difference in heavy chains between the four-chain γA and the two-chain γA is a set of five peptides indicated in Fig. 2. These five peptides were reproducible features of all the tryptic peptide maps prepared from heavy chains from Adj. PC-6A and MOPC 209B proteins and were not seen in either of the heavy-chain maps of the two-chain γA's shown or in maps of heavy chains isolated from the two-chain γA's of MOPC 88 and MOPC 116. The presence of these peptide spots was associated with the antigenic specificities of four-chain γA's. Further, these specificities have been localized on the papain Fc-fragment of the heavy chain (*9*). Absence of these peptide spots results in a loss of these specificities and the appearance of a new set of specificities shared by all two-chain γA's (class specificity of two-chain γA's). It would appear that the heavy chains of the two-chain γA's lack a structural unit which may be a prerequisite for larger forms of γA molecules. However, covalently bound carbohydrate or polypeptide and tertiary configuration have not been conclusively eliminated as the source of these peptide and antigenic differences.

The existence of an intermediate in the assembly of four-chain immunoglobulin molecules has been proposed for γG and γH immunoglobulins (*10*). Probably there is such an intermediate in the synthesis and assembly of γA immunoglobulins, and the two-chain molecules described in this paper are the tangible evidence for their existence. Some error in the protein assembly process of these few plasma-cell tumors seems to have prevented the completion of the molecule beyond a two-chain stage—normally a very transient and probably totally intracellular stage.

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Production of Ethylene by Fungi

Abstract. *Ethylene was detected by gas chromatography, and verified by chemical means, as a metabolic product of 22 species of fungi. Because 58 of 228 species of fungi produced a gaseous compound with retention time identical to that of authentic ethylene, we believe that this compound is a common metabolic product of fungi.*

Ethylene, a natural regulator of plant growth, is ubiquitous among higher plants (*1*) and is also produced by subcellular particles from rat tissues (*2*). Among fungi, however, the production of ethylene has been convincingly demonstrated by only three species, *Penicillium digitatum* (*3*), *Blastomyces dermatitidis* (*4*), *and Agaricus campestris bisporus* (*5*). The production of ethylene by baker's yeast (*6*) and *Fusarium oxysporum* f. *lycopersicum* (*7*) has been questioned (*1*). We know of no comprehensive survey of ethylene production by fungi; we now present evidence that ethylene is produced by many fungi.

Fungi were cultured in 500-ml erlenmeyer flasks containing 90 ml of medium (Staley's corn steep liquor, 40 g; technical glucose, 40 g; $CaCO_3$, 3.5 g; $NaNO_3$, 3.0 g; K_2HPO_4, 0.5 g; $MgSO_4$, 0.25 g; and deionized water, 1000 ml). After inoculation, the flasks were placed on a reciprocating shaker (99 to 100 cycle/min with 7.6-cm strokes) at 28°C for 6 to 8 days. The cultures were filtered through Whatman No. 1 filter paper, and the mycelium was used directly. Usually, only submerged pellets were used, but in some cases it was necessary to use mycelium adhering to the walls of the flask. Large pellets and compacted mycelium were sliced into sections (1 to 2 mm^3). Fungi which grew as single cells were centrifuged at 2000*g* for 5 minutes and decanted to remove culture filtrate. The fungi, approximately 4 ml (0.1 to 0.2 g, dry weight) were incubated in 10-ml glass syringes for 24 hours. Although ethylene was detected from many fungi after 5 hours, the longer period increased the

Table 1. Summary of fungi tested for ethylene production.

Major groups	Genera tested (No.)	Species tested (No.)
Phycomycetes		
Mucorales	15	31
Ascomycetes		
Endomycetales	2	2
Eurotiales	7	116
Sphaeriales	5	9
Hypocreales	2	5
Pezizales	2	3
Helotiales	2	2
Basidiomycetes		
Agaricales	6	6
Fungi imperfecti		
Sphaeropsidales	2	2
Melanconiales	2	2
Moniliales	35	49
Mycelia-Sterilia	1	1