*Summary.* On asynchronous and synchronized cultures of Chang's conjunctiva cells it was shown that cytotoxic antibodies (in the presence of complement) were most effective during the stage of cytoplasmic cell division, *i.e.,* between mitosis and $G_1$-phase. All other stages of the cellular life cycle were relatively resistant in that longer incubation periods were required for cell destruction. A newly developed combined tetrazolium-Feulgen reaction was used in these studies with advantage.

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## Subgroups of γA Immune Globulins.* (31287)

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Recently it has been demonstrated that at least 4 heavy chain subgroups exist for γG globulin(1,2). These differ in a variety of properties in addition to the antigenic properties utilized in their classification(3). Perhaps most striking is the independence of genetic factors for each subgroup(4). The possibility that γA and γM, the other 2 major classes of immune globulins, possess analogous subgroups has been considered by a number of investigators. Some evidence for such a possibility has been obtained for the γM macroglobulins(5,6). However, no findings of a similar nature have been reported for the γA type. The present report indicates that the γA globulins can be classified into 2 distinct types based on marked differences in the heavy chains.

*Material and methods.* Thirty-two myeloma proteins of the γA type were utilized in this study. Most of these came from sera sent to this laboratory for diagnostic typing. In addition, 10 of the proteins came from patients studied at the Clinic and Hospital of The Rockefeller University.

Six different antisera were utilized. Two were made in cynomologous monkeys (Mc and Ms) and 4 were made in rabbits ($R_2$, $R_c$, $R_4$, $R_5$). Antisera Mc and Rc were made against the same γA protein (c). This came from an individual who showed a sharp γA band over a period of 10 years with no evidence of myeloma. The remaining animals were immunized with separate γA myeloma proteins. In addition, 4 rabbits were immunized with isolated γA proteins of the minor subgroup, 2 with Ha and 2 with Os. The γA antisera were usually absorbed with serum Ro which was previously shown to be entirely devoid of γA protein(7).

The techniques utilized for protein isolation, separation of the heavy and light chains and agar gel analysis were similar to those described previously from this laboratory(1).

*Results. Subdivision of γA proteins into 2 types by antigenic analysis.* In the course of classification of a myeloma protein from a new patient under study, it was noted that this protein was clearly γA in type with one γA antiserum but difficult to type with another γA antiserum. Further studies indicated that several γA antisera showed this protein to be deficient antigenically. A survey was then instituted of 32 γA myeloma proteins and two others were found which behaved identically to the initial protein.

Fig. 1 illustrates the lines formed by agar plate analysis for 6 isolated γA proteins with

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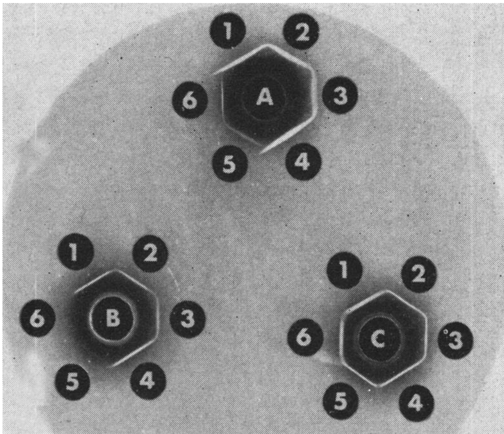


FIG. 1. Illustration of the bands formed by 6 isolated γA myeloma proteins with 3 different absorbed antisera (A, B, C in central wells). The antisera in A and B recognize proteins Ha and Os (wells 5 and 6) as deficient. The antiserum in C fails to distinguish γA proteins.

3 different γA antisera. Included in the 6 were 2 of the deficient type (Ha and Os). The antiserum in well C ($R_5$) gave a reaction of identity for all 6 γA proteins. However, the antiserum in well A (Mc) showed proteins Ha and Os to be markedly deficient and to give a reaction of identity with each other. Antiserum $R_4$ in well B gave a similar picture with the proteins Ha and Os showing only a very faint line. Further studies with an additional antiserum ($R_2$) showed the same proteins to be deficient (Fig. 2). Exactly similar findings were obtained with the third deficient protein. Six antisera were eventually studied and 3 of these showed the distinct differences between the γA proteins; 3 others showed a reaction of identity for all of the proteins and failed to distinguish the two types. Antisera $R_4$ and $R_5$ were made against the same protein in 2 different rabbits. The one antiserum showed the difference and the other failed to distinguish the γA proteins (Fig. 1). Most of the above work was carried out with absorbed antisera. However, the antigenic deficiency was also evident with the unabsorbed antisera.

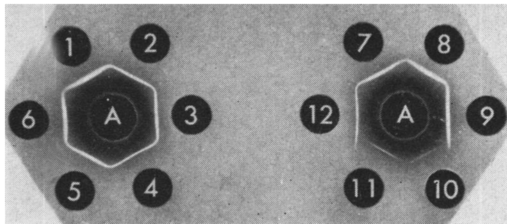


FIG. 2. Reaction of 12 different isolated γA proteins with another antiserum (in wells A) which recognizes proteins Ha and Os (in wells 10 and 11) as deficient.

Attempts were made to obtain specific antisera against the deficient γA proteins in an effort to demonstrate the difference in the reverse fashion. However, all efforts in this direction failed. Four animals were immunized with 2 of the deficient proteins over a considerable period but the antisera did not bring out new antigens in the deficient proteins.

*Chain localization of the antigenic differences.* The 3 deficient proteins all belonged to type κ (Group I) in respect to the light chain classification and the majority of the complete type also belonged in this category. This made it improbable that the light chains were involved in the distinction, particularly since the antisera were absorbed with γG globulin in most instances. Isolation of the heavy chains from 2 proteins of the complete type and one of the deficient demonstrated that the heavy chains were indeed responsible for the antigenic differences. The isolated heavy chains from the complete type spurred over those of the deficient group. In addition, pepsin digestion of the myeloma proteins which is thought to act specifically on the Fc fragment of the heavy chain of these proteins eliminated all reactivity with these absorbed antisera.

*Correlation with other properties.* It became apparent early in the investigation that the antigenic deficiency correlated in a general way with electrophoretic mobility. Fig. 3 illustrates in immunoelectrophoresis experiments the fast mobility to the anode of 2 of the deficient proteins as contrasted to one protein of the complete group. A few proteins of the complete group also showed similar fast mobility so that this characteristic was not an absolute distinguishing feature.

The heterogeneity of γA myeloma proteins by ultracentrifuge analysis is well known(7). The possibility arose that the deficient group might represent a particular molecular species.

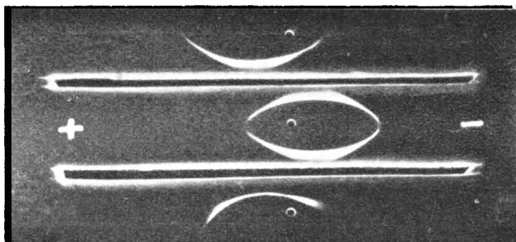


FIG. 3. Immunoelectrophoresis patterns illustrating the fast mobility toward the anode of proteins Ha (top well) and Os (bottom well) as contrasted to the slower mobility of a representative protein of the major type (middle well). Non-discriminating antiserum $R_5$ was used in each trough.

Studies with 2 of the isolated deficient proteins indicated that they were quite homogeneous with an s rate of approximately 7S. The $S^0_{20,w}$ for protein Ha was 6.8 S. However, a number of the complete type were very similar in ultracentrifuge properties. One of these, protein c, which was studied extensively and used for immunization of 2 of the animals was also very homogeneous and had an s rate indistinguishable from one of the deficient proteins in an experiment where both proteins were sedimented together in separate cells. It was apparent that the antigenic differences were not related to sedimentation properties.

*Discussion.* Three different antisera, one from a cynomologous monkey and 2 from rabbits, all served to classify 3 or 32 myeloma proteins as a distinct subgroup of γA globulin. These antigenic differences were quite striking and appeared to be greater than those previously described for the γG proteins where considerable immunization was usually required for preparation of discriminating antisera. No other clear antigenic differences between these proteins were encountered which might indicate additional subgroups although considerable further work with new antisera is essential to answer this question conclusively.

The 3 proteins forming the minor subgroup were only recognized as antigenically deficient. Antisera made against these proteins failed to bring out antigenic determinants specific for the subgroup. This was somewhat surprising since this was possible with the γG subclasses. As a consequence of this failure, studies of similar proteins among the γA globulins of normal serum were very restricted. Normal γA globulin behaved entirely analogous to the major type of myeloma proteins. However, if, as expected, the minor subgroup represented a minority of the γA globulin molecules in normal serum it would not be detected. A number of preparations of isolated γA globulin from various sources including ascitic fluid, saliva and colostrum all showed the major type as the dominant component. Previous studies have demonstrated a distinct relationship between the incidence of a specific type of myeloma protein among different patients and the molecular incidence of such a type among normal γ-globulin molecules(9,10). This would suggest that less than 10% of the γA molecules would belong to the minor subgroup, an amount which would be very difficult to detect without a specific antiserum.

The unusual electrophoretic mobility of the 3 proteins in the minor subgroup is of some interest. The possibility certainly exists that they are richer in carbohydrate than the bulk of γA proteins particularly with regard to sialic acid which influences the charge very significantly in the case of many serum proteins. A correlation of γG subgroups and electrophoretic mobility has also been observed. The Ge (γ2d) and Ne (γ2a) subgroups are in general more rapid in mobility than the other classes although exceptions are found. Also in the case of guinea pig and mouse γ-globulin, the γG subgroups are in most instances related to mobility(11).

The significance of the minor subgroup of γA globulin remains to be determined. Past experience has demonstrated repeatedly that observations of this type with myeloma proteins became significant in studies of antibody properties. In the case of the γG globulins each of the heavy chain subgroups contain different genetic factors and appear to be under separate genetic control. Such physiological properties as complement fixation and passive cutaneous anaphylaxis also have shown differences. Considerable controversy exists regarding the relationship of γA antibodies to reaginic antibodies(12) and per-

haps the minor subgroups hold significance in this relationship.

*Summary.* Three γA myeloma proteins were encountered which were very similar but differed markedly in antigenic properties from the bulk of γA proteins. Several antisera from rabbits as well as primates served to distinguish these proteins as belonging to a well defined subgroup. The distinguishing characteristics were shown to be properties of the heavy chains. A general but not absolute relationship to electrophoretic mobility was demonstrated.

ADDENDUM. Exchange of γA proteins with Drs. Vaerman and Heremans and also with Drs. Feinstein and Franklin indicate that these two groups of workers have also observed the same subgroups.

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## Sulfide Liberation from Raw Soybean Protein. (31288)

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Numerous reports have been concerned with the factors or properties that cause an inferior nutritive value of raw soybean meal as compared to properly cooked meal. These reports have been reviewed recently(1). Among such factors have been anti-enzymes, pancreatic hypertrophy, amino acid deficiency or unavailability, toxic proteins, and indigestible protein fractions, but the effects or even the existence of some are still matters of disagreement.

Proteins which are known to be highly indigestible, *i.e.*, the keratins, have been shown to be improved in digestibility by steam cooking under pressure. A comprehensive report (2) has reviewed the literature in this field and presented specific data in respect to feather protein. It was shown that the principal changes on treatment with steam were loss of cystine, corresponding appearance of lanthionine and increased susceptibility to enzymatic hydrolysis. The conversion of cystine in part to lanthionine, which involves breakage of disulfide cross links between amino acid chains and loss of one-half the sulfur, was most rapid during the first 30 minutes of processing at 30 lb steam pressure. At the same time, amino nitrogen liberated by rupture of peptide bonds increased very slightly, if at all.

Some data(3) obtained during steam processing of hoof and horn meal are in agreement with the later studies on feather protein. Volatile sulfur, particularly $H_2S$, was detected in the exhaust gases. Neartly two-thirds of the original cystine was lost, while the apparent digestibility to pepsin and HCl improved from an original 13 to 73%. The loss of sulfur