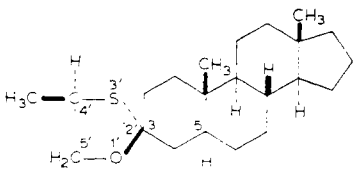
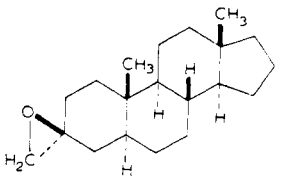
5. Spiro Derivatives. Spiro derivatives of steroids are named n accordance with the principles laid down in IUPAC Rules A-41, A-42, B-10, and B-11. Additional stereochemistry due to the spiro junction and substituents in the nonsteroid ring is designated by the sequence-rule procedure. Alternative names permitted by IUPAC rules are illustrated for (122) and (123).



(122)

4′*R*-Methyl-(*R*)-spiro[5α-androstane-3,2′-(1′,3′-oxathiolane)] or 5α-androstane-3(*R*)-spiro-2′-(4′*R*-methyl-1′,3′-oxathiolane)



(123)

(3*S*)-Spiro[5α-androstane-3,2′-oxiran] or (3*S*)-5α-androstane-3-spiro-2′-oxiran

Acknowledgment

We are indebted to the editors and publishers (Elsevier Publishing Company) of *Biochimica et Biophysica Acta* for permission to reproduce photographically the chemical structures that appeared in their publication of these tentative rules.

Immunochemical Studies on Tobacco Mosaic Virus Protein. IX. Investigations on Binding and Antigenic Specificity of Antibodies to an Antigenic Area of Tobacco Mosaic Virus Protein*

E. Benjamini, M. Shimizu, J. D. Young, and C. Y. Leung

ABSTRACT: Previous reports from this laboratory showed that antibodies produced by all rabbits in response to immunization with tobacco mosaic virus protein bind with a decapeptide representing residues 103–112 of the protein and having the sequence Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg. It was further shown that whereas no binding could be demonstrated between antibodies and the C-terminal di-, tri-, or tetrapeptide, antibodies produced by some rabbits bind with the C-terminal pentapeptide while those produced by other rabbits require the C-terminal hexa- or heptapeptide portions of the decapeptide for demonstrable binding. A subsequent report from this laboratory showed that N octanoylation of the C-terminal tetrapeptide conferred activity upon the peptide.

Using *N*-octanoylpeptides the present work demonstrates that antibodies produced by all the rabbits tested bind with the octanoylated C-terminal tripeptide portion of the decapeptide, namely with octanoyl-Ala-Thr-Arg. It is postulated that the differences which exist between antibodies produced by different rabbits with respect to reactivity with peptides consisting of C-terminal portions of the decapeptide are due to differences in the binding affinities with these peptides. In addition, experiments with closely related octanoylated analogs of the above tripeptide indicated differences between antibodies produced by different animals which are probably due to differences in antibody specificity.

It has been reported from this laboratory that the immunization of rabbits, guinea pigs, mice, and rats with tobacco mosaic virus protein elicited in all the animals, antibodies which bind with a tryptic eicosapeptide, representing residues 93–112 of the protein, and that all the rabbits which were tested produced antibodies which bind with the C-terminal decapeptide portion of the eicosapeptide, having the sequence Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg (Benjamini *et al.*, 1968a). We have also shown that whereas binding between the C-terminal di-, tri-, or tetrapeptide and anti-TMVP produced by all rabbits could not be demonstrated, some rabbits produced antibodies which bind with the C-terminal pentapeptide portion of the decapeptide, having the sequence Leu-Asp-Ala-Thr-Arg (Young *et al.*, 1967), while antibodies produced by other rabbits required the C-terminal hexa- or heptapeptide for demonstrable binding (Benjamini *et al.*, 1968a). In spite of these apparent differences in antibody populations among the various rabbits, all the animals produced antibodies which bind with peptides containing the C-terminal pentapeptide sequence Leu-Asp-Ala-Thr-Arg; it was therefore postulated that this sequence or part of it constitutes a portion of, or contains an area which is antigenic to all the rabbits tested.

Utilizing globulins containing antibodies which bind with the pentapeptide Leu-Asp-Ala-Thr-Arg, Young *et al.* (1968) pointed out that the amount of pentapeptide associated with these globulins could be roughly correlated with the degree of

* From the Laboratory of Medical Entomology, Kaiser Foundation Research Institute, and the Allergy Research Division, Allergy Department, Kaiser Foundation Hospital, San Francisco, California 94115. These studies were supported in part by U. S. Public Health Service Grant No. AI06040.

TABLE I: Amino Acid Analysis of [^{14}C]Octanoyl Peptides.

Peptide	Molar Ratio						
	Ala	Thr	Arg	Leu	α-ABu[a]	Ser	Gly
Octanoyl-Ala-Thr-Arg	0.99	1.00	1.00				
Octanoyl-Gly-Thr-Arg		1.05	0.96				0.99
Octanoyl-Leu-Thr-Arg		0.99	1.01	0.71			
Octanoyl-α-ABu-Thr-Arg		1.02	0.98		0.99		
Octanoyl-Ala-Gly-Arg	0.82		1.11				1.06
Octanoyl-Ala-Ser-Arg	1.02		1.00			0.98	
Octanoyl-Ala-Leu-Arg	1.00		1.03	0.97			
Octanoyl-Ala-α-ABu-Arg	1.06		0.97		0.98		
Octanoyl-α-ABu-α-ABu-Arg			0.97		2.03		

[a] α-ABu, α-aminobutyryl.

hydrophobicity of the N-terminal portion of the peptide. Subsequently it was reported (Benjamini *et al.*, 1968b) that the octanoylation of the amino group of the tetrapeptide Asp-Ala-Thr-Arg resulted in an octanoyl peptide which binds with these globulins. The amount of the octanoylated peptide associated with globulins was much higher than that of the non-octanoylated pentapeptide Leu-Asp-Ala-Thr-Arg; binding between these antibodies and the nonoctanoylated tetrapeptide could not be demonstrated. Furthermore, it was reported in the same communication that the octanoylated C-terminal tripeptide of the pentapeptide, namely *N*-octanoyl-Ala-Thr-Arg, bound specifically with antibodies whereas no binding could be detected with the nonoctanoylated tripeptide Ala-Thr-Arg; no binding could be demonstrated between antibodies and *N*-octanoyl-Thr-Arg.

In view of these findings it was of interest to examine sera produced by various rabbits in order to ascertain whether or not octanoyl-Ala-Thr-Arg and closely related octanoylated analogs would bind with antibodies which bind with the decapeptide, but not necessarily with its C-terminal pentapeptide. The present communication reports the results of these studies.

Experimental Section

Synthesis of Peptides and of Octanoylated Peptides. Peptide-resins were synthesized by the Merrifield solid-phase peptide synthesis method (Merrifield, 1964) as described by Stewart *et al.* (1966) and by Young *et al.* (1967). Octanoylation of peptides was performed with [^{14}C]octanoic acid as described by Benjamini *et al.* (1968b) using approximately 20 mg of peptide-resin. Following cleavage from the resin the octanoylated peptides were purified by passage through a G-10 Sephadex column (0.8 × 110 cm) equilibrated and eluted with collidine–pyridine–acetic acid (pH 8.0) buffer (20:20:0.75 ml made up to 1 l. with water) and by elution from electrophoretograms performed at pH 6.4 (Stewart *et al.*, 1966). Acetylated peptides were prepared and purified as previously described (Young *et al.*, 1967). The purity of each peptide was ascertained by electrophoresis and by amino acid analysis. Each peptide was electrophoresed at pH 6.4 as described by Stewart *et al.* (1966), sprayed with a peptide reagent (Nitecki and Goodman, 1966), stained for arginine with the Sakaguchi reagent, and scanned for radioactivity as previously described (Benjamini *et al.*, 1965). All preparations consisted of a single radioactive spot coinciding with a single Sakaguchi-positive or peptide-positive material, indicating purity. Each peptide was taken up in water, aliquots were hydrolyzed under open reflux in 6 N HCl at 110° for 24 hr, and analyzed for amino acids using the Spinco Model 120B amino acid analyzer. Results of the analyses shown in Table I closely agree with the theoretical values for the peptides, except for octanoyl-Leu-Thr-Arg where the value for leucine is low. In view of the fact that no ninhydrin-positive material was detected, this preparation probably contains some octanoyl-Thr-Arg which can not be resolved from octanoyl-Leu-Thr-Arg by the Sephadex and the electrophoretic conditions. The radioactivity of each *N*-acetyl or *N*-octanoyl peptide was measured in the Nuclear-Chicago Mark I liquid scintillation system using 1 μl of peptide in water, 2 ml of NCS reagent (Nuclear Chicago Corp., Des Plains, Ill.), and 12 ml of Omnifluor (New England Nuclear Corp., Boston, Mass.) in toluene (4 g of Omnifluor in 1 l. of toluene). The specific radioactivity of the [^{14}C]acetyl and octanoyl peptides was approximately 1.2×10^7 cpm/μmole.

Immunological Assay. Immunization with TMVP was performed as previously described (Benjamini *et al.*, 1965). Antisera were obtained from a single bleeding of each rabbit approximately 1 year following the initial injection. The rabbits used in these experiments were randomly bred. IgG was prepared according to the method of Levy and Sober (1960) using DEAE-cellulose column chromatography. The IgG fraction was made up in borate–saline buffer (pH 8.0) to a concentration of 4.5 mg/ml. Antilysozyme IgG at identical concentrations was used as control. The assay for immunological activity of the radioactive peptides (as described by Benjamini *et al.*, 1965) was performed using 10 mμmoles of peptide and 0.5 ml of globulins in a total volume of 0.6 ml and precipitation of the globulin–peptide complexes at 50% saturation of ammonium sulfate. After washings, the precipitates were dissolved in 1 ml of saline and aliquots of 0.3 ml were mixed with 2 ml of NCS reagent and 12 ml of Omnifluor in toluene. The number of counts bound was corrected for the specific activity of each peptide, using [^{14}C]octanoyl-Ala-Thr-Arg as the standard. The specificity of binding of each assayed

TABLE II: The Binding of Antibodies Produced by Various Rabbits with [^{14}C]Acetylated and [^{14}C]Octanoylated Peptides.

Peptide	Rabbit Number																			
	31,500			32,600			33,700			31,400			34,100			34,200			37,800	
	Cpm Bound[a]	Act.[b]	Specificity[c]	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity
Acetyl decapeptide	1,650	1.00	0.93	987	1.00	0.93	572	1.00	0.82	1,290	1.00	0.90	310	1.00	0.70	1,240	1.00	0.80	660	1.00	0.94
Acetyl pentapeptide	1,252	0.76	0.87	63	0.06	0.54	274	0.48	0.72	365	0.28	0.72	83	0.27	0.43	532	0.43	0.81	N.D.[d]	N.D.	N.D.
Octanoyl tetrapeptide	1,785	1.08	0.82	735	0.74	0.82	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	640	0.99	1.00
Octanoyl tripeptide	2,670	1.62	0.75	1,170	1.19	0.85	630	1.10	0.75	2,560	1.98	0.89	706	2.28	0.75	4,420	3.56	0.96	885	1.34	0.70
Octanoyl dipeptide	0	0	0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	0	0.00	33	0.11	0.07	0	0	0.00	N.D.	N.D.	N.D.

[a] Net counts per minute bound to 0.5 ml of anti-TMVP, average of two separate determinations. [b] Activity related to [^{14}C]acetyl decapeptide as 1.00. [c] Specificity defined as: 1 − (cpm bound to antilysozyme/cpm bound to anti-TMVP). [d] N.D., not done.

peptide was derived by computing the ratio of the number of counts bound with antilysozyme over the number of counts bound with anti-TMVP and substracting the value from unity (Benjamini *et al.*, 1968b).

The experiment with results presented in Table II was performed two times with duplicate countings except for assays of globulins obtained from rabbit 37,800 which were performed once. The experiment with results presented in Table III was performed three times with duplicate countings. Although variations in the number of counts bound to antibodies was noticed among assays performed at different times, the ratios of various peptides bound with the same antibody preparation remained relatively constant (±10%).

Results and Discussion

Data in Table II show the amounts of radioactive [^{14}C]-acetyl and of [^{14}C]octanoyl peptides bound to anti-TMVP produced by several rabbits. The data show that antibodies produced by all rabbits bind specifically with [^{14}C]acetyl decapeptide but that not all antibodies produced by the different rabbits bind significantly with the [^{14}C]acetyl pentapeptide. The data show that the amount of acetyl pentapeptide associated with antibodies produced by a given rabbit is not necessarily correlated with the amount of acetyl decapeptide associated with the antibodies produced by the same rabbit. Thus, whereas only 572 cpm of acetyl decapeptide was associated with antibodies produced by rabbit 33,700 which bind 274 cpm of acetyl pentapeptide, 987 cpm of acetyl decapeptide was associated with antibodies produced by rabbit 32,600 which bind only 63 cpm of acetyl pentapeptide.

Confirming previously published work (Benjamini *et al.*, 1968b) the enhancement of association with antibodies of peptides to which a strong hydrophobic group is attached N terminally is apparent in data presented in Table II. The data demonstrate that the rabbits tested produced antibodies which bind with octanoyl-Asp-Ala-Thr-Arg and furthermore, that all the rabbits produced antibodies which bind with octanoyl-Ala-Thr-Arg, although insignificant specific binding was observed between antibodies produced by two of these rabbits (32,600 and 34,100) and acetyl pentapeptide. In every instance the amount of octanoyl-Ala-Thr-Arg bound with antibodies was higher than that of acetyl decapeptide; the undetectable binding of antibodies, produced by all rabbits, with octanoyl-Thr-Arg is apparent in the data, and is in agreement with data previously reported (Benjamini *et al.*, 1968b).

Through the enhancement of association by the hydrophobic octanoyl group, data presented in Table II show that all the rabbits tested produced antibodies directed against Ala-Thr-Arg. Although the role of the hydrophobic area is still not clear, it has been suggested (Metzger *et al.*, 1963; Benjamini *et al.*, 1968b) that it enhances a possible hydrophobic interaction between the antigenic area and antibodies. This implies that the antibody site contains hydrophobic areas which interact with the hydrophobic area(s) of, or in proximity to the antigenic determinant. However, since the hydrophobic area of the antigen does not require any structural restrictions (it can consist of leucine, isoleucine, tyrosine, pentaalanine, acetyl, or octanoyl), it is conceivable that its interaction with the hydrophobic area on the antibody molecule is essentially nonspecific, and serves as an auxiliary mechanism to enhance the binding between the specific, sterically complementary areas

TABLE III: The Binding of Antibodies Produced by Various Rabbits with [^{14}C]Octanoyl-Ala-Thr-Arg and Octanoylated Analogs.

	Rabbit Number											
	33,700			31,400			34,100			34,200		
Peptide	Cpm Bound[a]	Act.[b]	Specificity[c]	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity	Cpm Bound	Act.	Specificity
Octanoyl-Ala-Thr-Arg	630[d]	1.00	0.75	1,920	1.00	0.89	975	1.00	0.78	6,030	1.00	0.91
Octanoyl-Gly-Thr-Arg	234	0.37	0.45	340	0.18	0.55	228	0.23	0.40	660	0.11	0.63
Octanoyl-α-ABu-[e]Thr-Arg	578	0.92	0.58	221	0.12	0.20	1,442	1.48	0.74	5,820	0.97	0.92
Octanoyl-Leu-Thr-Arg	316	0.50	0.30	148	0.08	0.17	438	0.45	0.41	578	0.10	0.58
Octanoyl-Ala-Ser-Arg	205	0.33	0.46	138	0.07	0.36	106	0.11	0.35	347	0.06	0.60
Octanoyl-Ala-Gly-Arg	172	0.27	0.35	40	0.02	0.14	23	0.02	0.11	145	0.02	0.34
Octanoyl-Ala-Leu-Arg	456	0.72	0.41	294	0.15	0.32	158	0.16	0.31	720	0.12	0.58
Octanoyl-Ala-α-ABu-Arg	290	0.46	0.43	13	0.01	0.03	118	0.12	0.33	330	0.05	0.57
Octanoyl-α-ABu-α-ABu-Arg	420	0.64	0.51	138	0.07	0.25	158	0.16	0.29	537	0.09	0.61

[a] Net counts per minute bound to 0.5 ml of anti-TMVP. [b] Activity related to [^{14}C]octanoyl-Ala-Thr-Arg as 1.00. [c] Specificity defined as: 1 − (cpm bound to antilysozyme/cpm bound to anti-TMVP). [d] Numbers represent averages of three separate determinations. [e] α-ABu, α-aminobutyryl.

on the antigen (Ala-Thr-Arg) and antibody sites, respectively. The only restriction imposed on this hydrophobic interaction is that the participating hydrophobic groups must be present in the correct position; the restriction is dictated by the juxtaposition of the antigenic sequence and the complementary antibody area to this sequence. The hydrophobic area of the antibody may be situated at or near the site complementary to antigenic specificity and thus contribute to the binding. This situation may be analogous to the recently suggested hydrophobic fold or slot located at or near the catalytic site of α-chymotrypsin, alkylated α-chymotrypsin, and alcohol dehydrogenase, which contributes to the binding of normal substrates (Royer and Canady, 1968).

If the above assumptions are correct, then differences in binding between various antibody populations directed against the same antigenic area of a protein may be due to differences in complementary hydrophobic areas of the antibody, in addition to possible differences in the abilities of the animals to recognize an area as antigenic or to synthesize antibody areas complementary to the antigenic areas. Data in Table II show drastic differences in the amounts of acetyl pentapeptide associated with antibodies produced by the different rabbits, although the antibodies produced by all the rabbits recognize the antigenic specificity Ala-Thr-Arg.

Having ascertained that antibodies produced by the different rabbits recognize the antigenic specificity Ala-Thr-Arg, the following question may be posed. Do antibodies, produced by different rabbits which recognize the same antigenic determinant possess the same complementary areas for antigenic specificity or do such areas differ from animal to animal? To elucidate this question, IgG of four rabbits which bind with octanoyl-Ala-Thr-Arg was tested for binding with octanoylated tripeptides in which alanine and threonine were replaced by other amino acids. This was done with the assumption that should all the antibodies possess identical antigenic specificity areas, the relative amounts of the various octanoylated analogs associated with antibodies would remain the same, regardless of the source of the antibodies. Data in Table III demonstrate that whereas the antibodies produced by all rabbits specifically bind with octanoyl-Ala-Thr-Arg, none of the antibodies exhibited high association of significant specificity with any of the octanoylated analogs except with octanoyl-α-aminobutyryl-Thr-Arg. With antibodies produced by three of the four rabbits tested, this analog was found to associate in amounts similar in magnitude or higher than did octanoyl-Ala-Thr-Arg, whereas very low amounts of α-aminobutyryl-Ala-Thr-Arg associated with antibodies produced by the fourth rabbit. These findings indicate that antibodies produced by different rabbits to the same antigenic determinant may have structurally different antigenic specificity sites.

Data presented in Table III demonstrate the high specificity of the combining sites of antibodies, produced by the different rabbits, toward Ala-Thr-Arg since even closely related octanoylated analogs of the peptide were inactive. Some peptides, notably octanoyl-Ala-Leu-Arg and octanoyl-α-aminobutyryl-α-aminobutyryl-Arg, exhibited relatively high binding with globulins produced by rabbit 33,700 but, since the binding was of relatively low specificity, it is difficult to define whether or not the antibodies are directed against these peptides. This demonstrates the difficulty in demarcation of antibody activity as has been pointed out by Burnet (1967). Since the inactive peptides were in the form of *N*-octanoyl, which is presumed to enhance association the lack of binding between a given peptide analog and antibodies is probably because structurally, the peptide analog cannot fit the antibody site which is directed against Ala-Thr-Arg. The finding that octanoyl-α-aminobutyryl-Thr-Arg binds with some antibodies better than octanoyl-Ala-Thr-Arg may mean that the antibodies which bind with the synthetic analog have a complementary area for antigenic specificity which shows a better fit to the analog than to the native sequence.

Acknowledgment

We wish to thank Dr. Ben F. Feingold for his interest throughout these studies. We also wish to thank Drs. D.

Michaeli and J. R. Kettman for their valuable advice and discussions. Thanks are extended to Mrs. S. San Juan for her superb technical assistance.

References

Benjamini, E., Shimizu, M., Young, J. D., and Leung, C. Y. (1968a), *Biochemistry 7*, 1253.

Benjamini, E., Shimizu, M., Young, J. D., and Leung, C. Y. (1968b), *Biochemistry 7*, 1261.

Benjamini, E., Young, J. D., Peterson, W. J., Leung, C. Y., and Shimizu, M. (1965), *Biochemistry 4*, 2081.

Burnet, F. M. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32*, 1.

Levy, H. B., and Sober, H. A. (1960), *Proc. Soc. Exptl. Biol. Med. 103*, 250.

Merrifield, R. B. (1964), *Biochemistry 3*, 1385.

Metzger, H., Wofsy, L., and Singer, S. J. (1963), *Arch. Biochem. Biophys. 103*, 206.

Nitecki, D. E., and Goodman, J. W. (1966), *Biochemistry 5*, 665.

Royer, G., and Canady, W. J. (1968), *Arch. Biochem. Biophys. 124*, 530.

Stewart, J. M., Young, J. D., Benjamini, E., Shimizu, M., and Leung, C. Y. (1966), *Biochemistry 5*, 3396.

Young, J. D., Benjamini, E., and Leung, C. Y. (1968), *Biochemistry 7*, 3113.

Young, J. D., Benjamini, E., Stewart, J. M., and Leung, C. Y. (1967), *Biochemistry 6*, 1455.

Studies on the Chymotrypsin C and Papain Fragments of Human Immunoglobulin M*

James P. Chen, Morris Reichlin, and Thomas B. Tomasi, Jr.†

ABSTRACT: Human Waldenstrom macroglobulins and their reductive subunits were subjected to proteolysis by chymotrypsin C (isolated from porcine pancreas) and papain. Chymotrypsin C cleaved immunoglobulin M into three fragments which had sedimentation coefficients of CI = 16.6 S, CII = 6.6 S, and CIII = 3.8 S. Antigenic analysis showed that CIII with the molecular weight of 40,000 corresponds to an Fabμ fragment. CII with the molecular weight of 135,000 is a dimer of CIII plus an additional fragment of 55,000. CI is a mixture which can be resolved by equilibrium ultracentrifugal technique into two major components with weight-average molecular weights of 773,000 and 606,000, and is composed of immunoglobulin M molecules from which one or more F(ab')$_2\mu$ fragments have been hydrolyzed. The calculation of the yield of Fabμ from proteolysis of the reductive subunit demonstrated the presence of 2 Fabμ units in the 7S subunits and 10 in the parent 19S immunoglobulin M. Short-term digestion of immunoglobulin M by papain produced three fragments which were separable on Sephadex gel filtration and had sedimentation coefficients of PI = 18.4 S, PII = 7.0 S, and PIII = 3.7 S. PIII contains two components corresponding to Fabμ and Fcμ fragments. After longer periods of incubation with papain, PIII (Fabμ fragments) with the molecular weight of 37,000 are the only immunologically reactive fragments detectable and the remainder of the molecule is degraded into peptides. It is believed that in the presence of 0.01 M cysteine immunoglobulin M is reduced to the reductive subunit of immunoglobulin M (PII) and then the 7S subunit is degraded into PIII by papain. PI is most likely the unreduced and undigested immunoglobulin M. Quantitative complement fixation techniques have been used to study the immunological relationships between the proteolytic fragments and to localize the antigenic determinants of various antisera including those responsible for the individual (idiotypic) specificity.

Nearly one-third of the total hexose found in the reductive subunit of immunoglobulin is bound to an F(ab')$_2\mu$ fragment. Hence, over two-thirds of the carbohydrate resides in the C-terminal region beyond the chymotrypsin C cleavage. The results of these studies are consistent with a circular pentameric model of immunoglobulin M containing 10 Fabμ units.

Miller and Metzger (1965) proposed that 19S IgM[1] molecules consist of five 7S subunits (IgMsb) which can be liberated by reduction and alkylation. This model is substantiated by quantitative studies involving molecular weights and yields of subunits (Miller and Metzger, 1965) and numbers of interchain disulfide bonds (Miller and Metzger, 1965) and is supported by recent electron microscopic evidence presented by Svehag *et al.* (Svehag *et al.*, 1967; Chesebro *et al.*, 1968).

Enzymatic degradative studies by Miller and Metzger (1966) using trypsin showed that tryptic hydrolysis of IgM yielded

* From the Department of Medicine, State University of New York at Buffalo, School of Medicine, Buffalo, New York 14203. *Received July 10, 1968.* Supported by National Institutes of Health Grant 2RO1AM10419 and National Institutes of Health Training Grant 5TO1AM05075. Presented in part at the 51st Meeting of the Federation of American Societies of Experimental Biology, Chicago, Ill.

† Address reprint requests to Buffalo General Hospital, Buffalo, N. Y. 14203.

[1] The abbreviations used are in accordance with the recommendations of the World Health Organization. The one not listed in *Bull. World Health Organ. 30*, 447 (1964) is: IgMsb, the reductive subunit of IgM.