These results demonstrate that in the rat the 1-hydroxy metabolite of 2-acetylaminofluorene arises largely, if not entirely, from the N-hydroxy metabolite, whereas the 5- and 7-hydroxy derivatives come largely, if not exclusively, from the parent amide. The intermediate activity of the 3-hydroxy metabolite indicates that some of this *o*-hydroxy derivative is derived from the N-hydroxy metabolite. Thus, while hydroxylation at the *para* site (7-) and certain of the *ortho* sites (3-, 5-) apparently involves a direct attack at these ring positions by activated oxygen[9], hydroxylation of the other *ortho* site (1-) apparently involves the intermediary formation and rearrangement of the N-hydroxy derivative, probably via the corresponding *o*-quinolimide (cf. ref. 5).

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A specific synthetic polypeptide antigen

All the specific antigens described till now are either natural substances (proteins, polysaccharides) or are derived from them by chemical modification. The large number of reported failures to detect antibodies against various synthetic homopolymers of α-amino acids suggests that the polymers studied are at the most very weak antigens[1]. Out of tens of synthetic polypeptides investigated by STAHMANN *et al.*[2,3] only one particular sample of poly-L-glutamic acid and a multichain copolymer of glutamic acid, leucine, glycine and lysine were reported to be antigenic. The antibodies formed did not precipitate with the homologous synthetic polypeptides, but cross-reacted with related polypeptidyl albumins as well as with various unrelated proteins. The synthetic polypeptides did not inhibit precipitin reactions between their antisera and proteins. MAURER *et al.*[4] reported recently that antibodies to a linear copolymer of glutamic acid and lysine could be detected by passive cutaneous anaphylaxis, but not by the precipitin reaction. Only tobacco-mosaic-virus protein inhibited the anaphylaxis reaction.

An investigation of the antigenicity of various polypeptidyl gelatins showed that, among others, the attachment of copolymeric chains of tyrosine and glutamic acid converted gelatin into a strong antigen whose specificity was due entirely to the polypeptides attached[5,6]. The question arises in this case whether gelatin still contributes in any way towards the immunological properties of the new antigen. In view of the above we have considered the possibility of replacing the gelatin with a synthetic material.

We have now been able to demonstrate that a multichain copolymer, in which chains of polypeptides containing L-tyrosine and L-glutamic acid were built on a multichain poly-DL-alanine[7,8] is a powerful and sharply specific antigen in rabbits. This copolymer was obtained by the polymerization in dioxane-0.05 *M* phosphate buffer (pH 7) (1:4), of N-carboxy-L-tyrosine anhydride and γ-benzyl-N-carboxy-L-glutamate anhydride, using the α-amino groups of multi-poly-DL-alanyl-poly-L-lysine[7,8] as polymerization initiators, followed by debenzylation with anhydrous hydrogen bromide; mol. wt. = 180.000; 9.9 % L-tyrosine residues; 15.3 % L-glutamic acid residues; 67.5 % DL-alanine residues; 7.0 % L-lysine residues.

The synthetic material was injected three times, at fortnightly intervals, in a complete adjuvant mixture into rabbits. The antibodies formed could be demonstrated by the usual precipitin reaction (Table I and Fig. 1). In order to determine directly the antigen in the specific precipitate, a preparation labelled with ^{131}I was used for precipitation. Except for the homologous antigen, the antibodies could be cross-precipitated only with the related copoly (tyrosyl, glutamyl) gelatin (Fig. 1). The homologous reaction could be inhibited completely by a copolymer of L-tyrosine and L-glutamic acid in a residue molar ratio of 1:1, only partially by a copolymer of these amino acids in a ratio of 1:4, and not at all by a copolymer of the same amino acids in a ratio of 1:9. Neither cross-reaction nor inhibition was obtained with linear poly-DL-alanine, multichain poly-L-glutamic acid, or unrelated proteins such as egg albumin or human γ-globulin.

Multichain poly-DL-alanine (the material which yielded the synthetic antigen after enrichment with tyrosine and glutamic acid), as well as a linear copolymer of L-glutamic acid and L-tyrosine in a residue molar ratio of 1:1 (degree of polymerization, 31), failed to produce antibodies detectable by the homologous precipitin test

TABLE I

COMPOSITION OF THE IMMUNOSPECIFIC PRECIPITATES

Antigen added per 1 ml serum (μg)	*Antigen precipitated* (μg)*	*Antigen precipitated (%)*	*Antibody precipitated** (μg)*	*Antibody/antigen in precipitate (w/w)*
12.5	12.35	99.0	195	15.4
31.2	26.5	85.0	321	12.1
62.5	47.2	75.5	418	8.9
125	70.0	56.0	468	6.7
250	80.8	32.3	412	5.1
437	79.3	18.2	345	4.3
750	77.5	10.4	260	3.4
1250	70.3	5.6	212	3.0

* From radioactivity data.

** From extinction at 2800 Å of neutralized solutions of the precipitates in NaOH, after deducting the calculated extinction of the antigen.

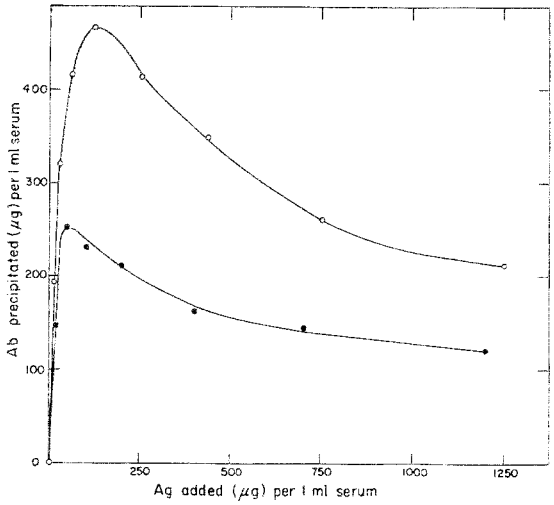

Fig. 1. Precipitin curves of copoly (tyrosyl, glutamyl) multichain poly-DL-alanine (○) and copoly (tyrosyl, glutamyl) gelatin (●) with the antiserum to copoly (tyrosyl, glutamyl) multichain poly-DL-alanine. The amounts of antibodies were obtained from extinction at 2800 Å after deducting the calculated extinction of the antigens. The amount of antigen in the precipitate was obtained for the synthetic material from the radioactivity data, and for the gelatin derivative from hydroxyproline data.

when injected similarly into rabbits. Nor could antibodies be detected by cross-precipitation with related polypeptidyl derivatives.

In view of the enhancement of the antigenicity of gelatin by attachment of tyrosine and other aromatic amino acids[5,6], and in view of the strong specific antigenicity of the tyrosinated synthetic multichain polypeptide, it seems that the potentiation of antigenicity by tyrosination may be of a more general character.

A study, now in progress, of the possible antigenicity of various other synthetic linear and multichain copolymers of α-amino acids should permit the elucidation of the role of molecular weight, shape, composition, and locus of specific amino acids, in conferring the antigenic properties on a molecule.

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