[56] Hassid, W. Z., E. W. Putman, and V. Ginsburg, *Biochim. et Biophys. Acta*, **20**, 17 (1956).
[57] Feingold, D. S., E. F. Neufeld, and W. Z. Hassid (in preparation).
[58] Feingold, D. S., E. F. Neufeld, and W. Z. Hassid, *Fed. Proc.*, **18**, 224 (1959); Neufeld, E. F., D. S. Feingold, and W. Z. Hassid, *Arch. Biochem. and Biophys.* (in press).
[59] Neufeld, E. F., D. S. Feingold, and W. Z. Hassid, *Am. Chem. Soc. Abstracts*, **60**, April 1959.
[60] Barker, S. A., E. J. Bourne, J. G. Fleetwood, and M. Stacey, *J. Chem. Soc.*, 4128 (1958).
[61] Altermatt, H. A., and A. C. Neish, *Can. J. Biochem. Physiol.*, **34**, 405 (1956).
[62] Neish, A. C., *Can. J. Biochem. Physiol.* **36**, 187 (1958).
[63] Seegmiller, C. G., R. Jang, and W. Mann, Jr., *Arch. Biochem. and Biophys.*, **61**, 422 (1956)
[64] Slater, W. G., and H. Beevers, *Plant Physiol.*, **33**, 146 (1958).
[65] Hassid, W. Z., and M. Doudoroff, *Advances in Enzymology*, **10**, 123 (1950).
[66] Charlson, A. J., and A. S. Perlin, *Can. J. Chem.*, **34**, 1200 (1956).
[67] Aspinall, G. D., and G. Kessler, *Chem. and Ind.*, London, 1296 (1957).
[68] Kessler, G., *Ber. schweiz, bot. Ges.*, **68**, 5 (1958).
[69] Eschrich, W., *Protoplasma*, **47**, 487 (1956).
[70] Currier, H. B., *Am. J. Botany*, **44**, 478 (1957).
[71] Calvin, M., *J. Chem. Soc.*, 1895 (1956).
[72] Bassham, J. A., and M. Calvin, *The Path of Carbon in Photosynthesis*, (New York: Prentice-Hall, 1957), 34–75.
[73] Roseman, S. (personal communication).

---

## *EFFECT OF SOLVENTS AND OF TEMPERATURE ON THE OPTICAL ROTATORY PROPERTIES OF PEPSIN**,†

BY GERTRUDE E. PERLMANN

THE ROCKEFELLER INSTITUTE

*Communicated by L. G. Longsworth, May 13, 1959*

In a current project in this laboratory, the chemical and physicochemical properties of pepsin are being investigated in an attempt to define the configuration of the peptide chain or chain segments that determines the biological activity of this enzyme. Inasmuch as a correlation exists between the rotatory properties and the configuration of proteins in solution, the optical rotation of pepsin has been examined in the presence and absence of such reagents as urea, guanidine, and lithium bromide which are known to alter the secondary structure of proteins. Although it has been previously reported by Simpson and Kauzmann and by Jirgensons that the specific rotation of pepsin, $[\alpha]_D$, does not change on short exposure to urea[1] or to guanidine,[2] a certain anomalous behavior, hitherto not yet described for other proteins, has been encountered and will, therefore, be reported here. Moreover, an attempt has been made to relate the optical properties to the enzymic activity of pepsin.

*Materials.*—The pepsin used in this research was the Worthington crystalline product, lot 623, with a nitrogen content of 14.6$_4$ per cent, the molar extinction coefficient, $\epsilon = 51.3_9 \times 10^3$ at $\lambda$ 278 m$\mu$ and the amino acid composition given elsewhere.[3]

Reagent grade urea and guanidine hydrochloride were recrystallized twice from 60 per cent ethanol and absolute methanol, respectively, whereas the lithium bromide was used without further purification.

*Optical Rotation.*—The optical rotation was measured in the wave length range

from 350 to 700 mμ with the Rudolph precision ultraviolet polarimeter, model 80, equipped with the photoelectric detector and oscillating polarizer prism. Although most of the determinations were made at 23°C with a Xenon Arc lamp as light source, in some experiments the temperature was varied from 15 to 65°C. Here, water from an external bath was circulated through jacketed cells and served to control the temperature to ±0.05°C. In these measurements the light source was a mercury lamp.

Since the indices of refraction of solvents containing urea, guanidine or lithium bromide differ considerably from water, all specific rotations given in this paper are corrected to the corresponding values in water with the aid of the equation[4]

$$[\alpha]_s = [\alpha] \frac{n^2_{H_2O} + 2}{n_s^2 + 2}$$

In each case the refraction of the solvent was measured with an Abbe refractometer.

*Protein Concentration.*—Protein concentrations in acetate buffers and lithium bromide were determined from nitrogen analyses performed upon the solutions by the Pregl micro-Kjeldahl method. The factor of 6.83 was used to convert the nitrogen values to dry weight. In the urea- or guanidine- containing solutions the optical density at 278 mμ served to estimate the content of pepsin.

*Enzyme Assay.*—All solutions were assayed routinely for enzymic activity by the hemoglobin method.[5] In some experiments, the hydrolysis of the synthetic substrate N-acetylphenylalanyl-dl-diiodotyrosine, kindly supplied by Dr. R. Pitt-Rivers, was also tested.

## RESULTS

*Effect of Urea and Guanidine.*—Previous investigations[1, 6] have shown that most globular proteins have a specific rotation, $[\alpha]_D$, in the range of $-30°$ to $-70°$ which on denaturation with urea or guanidine salts becomes more negative by 20° to 60°. Similarly, the optical rotatory dispersion constant, $\lambda_c$, is lowered from the range of 230–270 mμ to 212 mμ after contact with these reagents.[7] As a result of these early investigations, and of a study of collagen and gelatin, Cohen suggested in 1955 that the optical rotatory properties could be correlated with the helix content of the protein peptide chain.[8] As an extension of this suggestion Yang and Doty[9] proposed that the optical properties of the two configurations of poly-γ-benzyl-L-glutamate, i.e., the α-helix and the random coil, suffice to explain the characteristics of proteins. Thus a $\lambda_c$ of 212 mμ is assigned to a protein in randomly coiled form devoid of any helical structure, whereas a higher value of $\lambda_c$ indicates that a certain fraction of the amino acid residues of the protein is in the helical configuration.

In our investigations of the rotatory properties of pepsin, it immediately became apparent that although the specific rotation at 600 mμ in 0.1 *N* acetate buffer of pH 3.6 to 5.6 is within the range of globular proteins, i.e. $[\alpha]_{600} = -63.5 \pm 0.5°$, the constant $\lambda_c = 216$ mμ is unusually low although agreeing well with that reported by Jirgensons.[10] In all the solvents tested thus far the dispersion follows a one-term Drude equation. Consequently, if the data are expressed as plots of $[\alpha]$ versus $[\alpha]$. $\lambda^2$, [9] a linear relationship is obtained over the spectral range of 350 to 700 mμ.

If pepsin is dissolved in 8.0 $M$ urea-acetate of pH $5.3_5$, the specific rotation and rotatory dispersion is the same as that found in the 0.1 $N$ acetate buffer of the same pH. If, as shown in Table 1, the protein is kept in contact with the solvent at

TABLE 1

SPECIFIC OPTICAL ROTATION AND ROTATORY DISPERSION OF PEPSIN IN UREA-ACETATE AND GUANIDINE HYDROCHLORIDE

| Composition of Solvent | pH* | [α] 600 mμ | [α] 400 mμ | $\lambda_c$ | Relative Specific Activity per Unit Nitrogen† in Per Cent |
|---|---|---|---|---|---|
| 0.1 $N$ acetate buffer | $4.6_4$ | $63._3$ | 178 | 216 | 100 |
| 8.0 $M$ urea-acetate | | | | | |
| 0 time | $5.3_5$ | $64._1$ | 179 | 218 | 95 |
| 6 hrs., 37°C | $5.3_5$ | $64._9$ | 175 | 209 | 74 |
| 24 hrs., 37°C | $5.3_5$ | $64._0$ | 168 | 202 | 10 |
| Urea removed by dialysis against acetate buffer | $4.6_4$ | $60._4$ | 164 | 210 | 66 |
| 3.0 $M$ guanidine hydrochloride | 3.4 | $62._1$ | 173 | 217 | 100 |
| 4.0 $M$ guanidine hydrochloride | $3.3_8$ | $59._7$ | 166 | 219 | 100 |
| 5.0 $M$ guanidine hydrochloride | | | | | |
| 0 time | $3.4_1$ | $60._8$ | 165 | 218 | 93 |
| 6 hrs., 23°C | $3.4_1$ | $60._8$ | 158 | 200 | 80 |
| 24 hrs., 23°C | $3.4_1$ | $56._1$ | 149 | 201 | 51 |

* Apparent pH.
† The relative specific activity of a freshly prepared pepsin solution in 0.1 $N$ acetate buffer of pH 4.6 is taken as 100.

37°C for an extended period, $[\alpha]_{600}$ remains unchanged but $\lambda_c$ decreases from 218 mμ to 202 mμ during a period of 24 hours. This change of $\lambda_c$ is not due to a helix-coil transition as one would expect if a great number of hydrogen bonds were broken. Rather the presence of urea enhances the rate of autodigestion which is accompanied by an apparent inactivation of the enzyme and the formation of dialyzable material.[11] Since the optical rotatory dispersion constant of low molecular weight peptides is lower than that of proteins, the $\lambda_c$ of 202 mμ, given in Table 1, reflects the contribution of these peptides and is superimposed on the value of $\lambda_c$ of the residual protein. On removal, by dialysis, of the low molecular weight material, the rotatory dispersion constant of the non-dialyzable fraction increases to 210 mμ, a value similar to that of the starting material. Furthermore, the residual non-dialyzable fraction has a high specific activity per unit of nitrogen. Although the value of $\lambda_c$ given above is still somewhat below that of pepsin it is due to the residual 34 per cent of inactive but nondialyzable components, a conclusion confirmed by electrophoretic fractionation of the nondialyzable material. It is thus clear that the changes described here do not originate from an alteration of the secondary structure but are probably a result of the autolysis process. A behavior similar to that described above is encountered if pepsin is dissolved in guanidine hydrochloride. As may be seen in Table 1, the presence of 3.0 $M$ guanidine hydrochloride does not affect the optical parameters, $[\alpha]$ and $\lambda_c$, nor the enzymic activity. In 5.0 $M$ guanidine hydrochloride, the specific rotation, $[\alpha]_{600}$, however, increases somewhat and $\lambda_c$ decreases from 216 mμ to 200 mμ during a 30-minute exposure to the reagent. If the rotatory properties of pepsin in this solvent are followed over a period of 24 hours, the specific rotation at all wave lengths becomes less negative without the value of $\lambda_c$ being affected. It has also been recognized recently that the presence of guanidine salts in the pH range of 3.0 to 5.0 enhances the rate of

autodigestion of pepsin.[12] At 3.0 *M* guanidine hydrochloride at 25°C, autolysis is slow but occurs at an appreciable rate if the concentration of the reagent or the temperature is raised.

From the results to date, one can conclude that pepsin solutions at 20° to 25°C do not contain appreciable amounts of the protein in the $\alpha$-helix form. Invariably if a lowering of $\lambda_c$ occurs in the presence of the so-called hydrogen bond breaking reagents, it coincides with the formation of low-molecular weight peptides that results from the autodigestion of the protein.

*Effect of Lithium Bromide.*—Harrington and Schellman have recently investigated the effect of concentrated lithium bromide on the optical rotatory properties of several proteins. In view of the fact that this reagent lowers the activity of water, these investigators suggest that in certain instances at least, lithium bromide may strengthen hydrogen bonds, i.e., favor helix formation. Such an effect should cause changes opposite to those usually observed with urea or guanidine. The specific rotation, $[\alpha]$, should be less negative whereas $\lambda_c$ should increase if compared with the values found in buffer solutions. Changes of this type were actually observed by Harrington and Schellman[4, 13] when ribonuclease, clupein, silk fibroin, and serum albumin were examined in solutions of lithium bromide.

Inspection of Table 2 reveals that addition of a given concentration of lithium

TABLE 2

SPECIFIC OPTICAL ROTATION AND ROTATORY DISPERSION OF PEPSIN IN LITHIUM BROMIDE AND AFTER REMOVAL OF LITHIUM BROMIDE BY DIALYSIS AGAINST 0.1 *N* ACETATE BUFFER, pH 4.6

| Composition of Solvent | pH* | $[\alpha]$ 600 mμ | $[\alpha]$ 400 mμ | $\lambda_c$ | Relative Specific Activity per Unit Nitrogen in Per Cent |
|---|---|---|---|---|---|
| 1.0 *M* LiBr-acetate | 4.4$_8$ | 62.$_6$ | 168 | 208 | 100 |
| 2.0 *M* LiBr-acetate | 4.3$_2$ | 61.$_3$ | 173 | 207 | 66 |
| LiBr removed† | 4.6$_4$ | 65.$_4$ | 184 | 218 | 100 |
| 3.0 *M* LiBr-acetate | 4.0$_8$ | 61.$_0$ | 169 | 205 | 40 |
| LiBr removed† | 4.6$_4$ | 66.$_3$ | 185 | 218 | 100 |
| 4.0 *M* LiBr-acetate | 3.9$_6$ | 56.$_7$ | 160 | 218 | 24 |
| LiBr removed† | 4.6$_4$ | 66.$_4$ | 161 | 215 | 100 |
| 5.0 *M* LiBr-acetate | 3.8$_8$ | 54.$_8$ | 154 | 219 | 18 |
| LiBr removed† | 4.6$_4$ | 67.$_4$ | 187 | 213 | 89 |

* Apparent pH.
† LiBr removed by dialysis against 0.1 *N* acetate buffer of pH 4.6.

bromide reduces the activity of the enzyme by an amount proportional to the salt concentration and that no further change occurs. The specific rotation, $[\alpha]_{600}$, is not altered appreciably up to 3.0 *M* lithium bromide. However, $\lambda_c$ decreases from 216 mμ to 205 mμ. In contrast to urea and guanidine, no increase of trichloroacetic acid soluble material occurs. On increasing the concentration of lithium bromide to 5.0 *M*, the highest concentration at which pepsin remains soluble in this reagent, the specific rotation is less negative and $\lambda_c$ increases from 205 to 218 mμ. In every instance removal of lithium bromide by dialysis restores the enzymic activity, measured either with hemoglobin or the dipeptide, N-acetyl-phenylalanyl-dl-diiodotyrosine as substrates. Moreover, the values of $[\alpha]$ and $\lambda_c$ approach those found for pepsin in acetate buffer in the pH range of 3.6 to 5.6.

Although these results are in apparent contrast with those of Harrington and

Schellman, and can be taken as an indication that no appreciable helix formation takes place in the case of pepsin, it should be pointed out that a phenomenon similar to the one described here has recently been noticed by Harrington with collagen and gelatin.[14]

*Effect of Temperature.*—Since at 25°C the optical rotatory properties of pepsin do not undergo changes if "hydrogen-bond breaking" or "hydrogen-bond strengthening" reagents are added to a pepsin solution, the effect of temperature on the optical rotatory properties has been investigated. A point of interest immediately emerges from Table 3. In 0.1 *N* sodium acetate buffer of pH 4.6, the specific rotation $[\alpha]_{546}$ and $[\alpha]_{365.8}$, respectively, increase slightly and $\lambda_c$ changes from 216 to 226 mμ, if the temperature is raised from 40 to 60°C. Throughout all these measurements, the enzyme remains fully active and no increase in nonprotein material as tested by the solubility of the protein in hot 10 per cent trichloroacetic acid occurs. Inactivation of the enzyme starts at temperatures above 60°C and the change of $[\alpha]$ and $\lambda_c$ precedes somewhat the loss of biological activity.

Similarly, if urea or guanidine hydrochloride are added, $\lambda_c$ also increases with temperature.. However, a marked loss of peptic activity has already occurred at 45°C. In no case could formation of low-molecular weight material be detected, thus excluding the influence of autodigestion on either the activity or on the altering of the optical properties of the protein as is the case below 40°C. Moreover, it should be pointed out that $d[\alpha]/dT$ is negative in the acetate buffer, but positive in urea and guanidine. Here again the behavior of pepsin is in direct contrast to that of other proteins, e.g., β-lactoglobulin.[13]

The data in Table 3 show the temperature dependence of the optical rotatory

TABLE 3

SPECIFIC OPTICAL ROTATION AND ROTATORY DISPERSION OF PEPSIN IN ACETATE, 8.0 *M* UREA-ACETATE, 5.0 *M* GUANIDINE HYDROCHLORIDE AND LITHIUM BROMIDE-ACETATE AS FUNCTION OF TEMPERATURE

| Composition of Solvent | pH* | Temperature in °C | [α] 546 mμ | [α] 365.8 mμ | $\lambda_c$ | Relative Specific Activity per Unit Nitrogen in Per Cent |
|---|---|---|---|---|---|---|
| 0.1 *N* acetate buffer | 4.3 | 23° | 78.6 | 232 | 218 | 100 |
| | | 50° | 77.6 | 232 | 227 | 99 |
| | | 55° | 75.9 | 228 | 228 | 103 |
| | | 60° | 75.1 | 221 | 226 | 100 |
| 8.0 *M* urea-acetate | 5.3 | 23° | 77.7 | 229 | 218 | 95 |
| | | 50° | 78.3 | 237 | 223 | 11 |
| | | 55° | 79.7 | 241 | 229 | 5 |
| | | 60° | 80.2 | 241 | 229 | 1 |
| | | 65° | 79.3 | 240 | 230 | 0 |
| 5.0 *M* guanidine hydrochloride | 3.5 | 23° | 76.4 | 224 | 218 | 100 |
| | | 45° | 75.5 | 222 | 220 | 5 |
| | | 50° | 77.8 | 226 | 220 | ... |
| | | 55° | 77.4 | 226 | 226 | 4 |
| 2.5 *M* LiBr-acetate | 4.1 | 20° | 75.6 | 223 | 205 | 100 |
| | | 40° | 76.1 | 225 | 219 | 103 |
| | | 45° | 77.7 | 226 | 220 | 104 |
| | | 50° | | | | 106 |
| | | 55° | not measured | | | 93 |
| 5.0 *M* LiBr-acetate | 3.6$_8$ | 25° | 69.2 | 204 | 218 | 88 |
| | | 37° | 69.0 | 202 | 223 | 73 |
| | | 45° | 65.4 | 198 | 230 | 33 |
| | | 50° | 63.3 | 198 | 235 | 2 |

* Apparent pH.

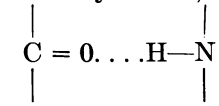
properties of pepsin in the presence of 2.5 *M* and 5.0 *M* lithium bromide. In 2.5 *M* lithium bromide $d[\alpha]/dT$ is positive and $\lambda_c$ increases from 205 m$\mu$ to 220 m$\mu$ if the temperature is raised to 50°C. The enzymic activity is not affected. In 5.0 *M* lithium bromide, on the other hand, $d[\alpha]/dT$ is negative. If the pepsin solution is kept at 37°C for 15 minutes appreciable inactivation can be detected, both if tested in the presence or absence of the reagent.

It can be concluded from these results that an increase of the rotatory dispersion constant, $\lambda_c$, from 216 to 226–230 m$\mu$, invariably precedes or accompanies the loss of the biological activity of the enzyme.

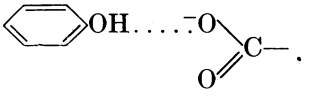
*Discussion.*—In the present investigation it has been shown that the enzymically active form of pepsin in solution is characterized by certain optical parameters, $[\alpha]$ and $\lambda_c$. In all solvents thus far examined, an increase of $\lambda_c$ coincides with an irreversible loss of activity. On the other hand, a lowering of $\lambda_c$, in most instances is indicative of the presence of low-molecular weight material that arises from autodigestion of the enzyme. In view of the peculiar properties of pepsin, the results of the optical rotatory measurements will be discussed in terms of the amino acid composition of the protein.

Pepsin, with a molecular weight of 35,000 is a protein with a single peptide chain[15] which consists of 343 amino acid residues and is cross linked by three disulfide bonds. From the low intrinsic viscosity of the protein, $\eta = 3.0_9$ gm. ml, it follows that the peptide chain is compactly folded. Moreover, the protein is unusual in that it has 71 acidic and only 4 basic amino acids and possesses an exceptionally high content of proline and of serine. Thus, it is quite likely that the low value of $\lambda_c = 216$ m$\mu$ is a reflection of the large amounts of glutamic, aspartic acid, and serine.[3]

The fact that on short exposure of pepsin to urea or guanidine in the pH range of 3.0 to 5.0 at 25°C, the optical rotatory properties remain unchanged and the enzyme is fully active, is of special interest. Although hydrogen bonds of the type



and those involving the phenolic hydroxyls of tyrosine and the carboxylate ions of the acidic amino acid residues, i.e.,



undoubtedly exist in pepsin[16] they must be relatively unimportant in maintaining the configuration necessary for the enzymic activity of this protein. Moreover, if the 18 proline residues are distributed statistically along the peptide chain only short helical segments could exist since the presence of the pyrollidine ring in the peptide chain prevents helix formation. Hence, one can assume that pepsin has a "random coil" configuration. Moreover, in view of the fact that hydrogen bond breaking reagents are ineffective in altering the secondary structure, other bonds such as hydrophobic bonds may play a role in stabilizing the molecule, a view further supported by the fact that 63 per cent of the amino acid residues are of nonpolar nature.‡

An alternative explanation for the "apparent lack" of an extensive helical con-

figuration follows from the fact the pepsin has 41 aspartic acid residues and 44 serines. Blout and Karlson[17] and Fassman[18] reported recently that the optical rotatory dispersion of β-benzyl-L-aspartate and of poly-L-serine differs markedly from that of poly-L-glutamate and other polyamino acids. The anomalous rotatory behavior of pepsin could, therefore, be explained if sequences of the type -Asp·Asp·Asp-. . .or -Ser·Ser·Ser- would be present in this protein and would counteract the contribution of the other amino acids to the "right-handed helix."

Although the decrease of $\lambda_c$ at low concentrations of lithium bromide distinguishes pepsin from the behavior of the globular proteins, such as ribonuclease, a similar change of the dispersion constant has recently been reported by Harrington and Sela[19] if poly-L-proline or proline-glycine copolymers are dissolved in lithium bromide. These investigators proposed that a trans-cis isomerization of the type described by Kurtz, Berger, and Katchalski[20] is mediated by lithium bromide. Although the occurrence of trans-cis isomerization has not yet been demonstrated in proteins, it is not unlikely that it may exist if the proline residues were present in sequences of the type -Pro·Pro-, -Pro·Pro·Gly- or -Gly·Pro·Pro- and may play a considerable role in biological systems.

A further point of interest is that unlike β-lactoglobulin, insulin and clupein[13] the optical rotatory dispersion constant, $\lambda_c$, of pepsin increases if the temperature is raised above 45°C and that this change coincides with or precedes the inactivation of the protein. This phenomenon may be due to the fact that certain amino acid residues, e.g., proline, lock the peptide chain into a certain configuration which confers considerable stability upon the protein. At higher temperatures, however, transition to a less stable configuration takes place. It is clear that in the case described here the chain segment necessary for the biological activity of the enzyme has been affected.

From the results presented in this communication, it is apparent that the application of the optical rotatory dispersion to the study of the secondary structure of proteins can be rendered even more powerful and unambiguous if supplemented by the use of other chemical and physicochemical methods.

*Summary.*—The effect of solvents and temperature on the optical rotatory dispersion of pepsin has been investigated. Urea and guanidine salts do not affect the specific optical rotation, $[\alpha]$, at 600 mμ. When a lowering of the rotatory dispersion constant $\lambda_c$ from 216 to 200 mμ occurs, it has been shown to be a consequence of autodigestion of the enzyme. At temperatures above 45°C an increase of $\lambda_c$ from 216 to 226–230 mμ takes place which coincides with a loss of the enzymic activity of pepsin. It may be concluded that the helical content of pepsin is negligible.

My sincere thanks go to Dr. Ephraim Katchalski for having introduced me to this type of work and to Dr. William F. Harrington of the National Institutes of Health for helpful discussion and for the hospitality of his laboratory where some of the measurements were carried out. The author is indebted to Mrs. Inese Leimanis for her able assistance in the activity measurements.

* This work was supported in part by Grants B-930 and A-2449 of the United States Public Health Service.

† A preliminary report of this work was presented at the Fourth International Congress of Biochemistry, Vienna, 1958; to be published in Symposium VIII, Pergamon Press.

‡ Pepsin is insoluble in hydrophobic bond-breaking reagents. Although it is possible to dissolve the protein in dichloroacetic acid, an irreversible loss of activity occurs instantaneously; likewise exposure to 2-chloroethanol inactivates pepsin whether the assay is conducted in the presence or absence of the reagent.

[1] Simpson, R. B., and W. Kauzmann, *J. Am. Chem. Soc.*, **75**, 5139 (1953).

[2] Jirgensons, B., *Arch. Biochem. Biophys.*, **39**, 261 (1952). Jirgensons, B., *ibid.*, **41**, 333 (1952).

[3] Blumenfeld, O. O., and G. E. Perlmann, *J. Gen. Physiol.*, **42**, 553 (1959).

[4] Harrington, W. F., and J. A. Schellman, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **30**, No. 12 (1957).

[5] Anson, M. L., in *Crystalline Enzymes*, ed. J. H. Northrop, M. Kunitz, and R. M. Herriott, editors, Columbia Biological Series, No. 12, (New York: Columbia University Press, 2nd edition, 1948), 305.

[6] Hewitt, L., *Biochem. J.*, **21**, 216 (1927).

[7] Linderstrøm-Lang, K., and J. A. Schellman, *Biochim. Biophys. Acta*, **15**, 203 (1955).

[8] Cohen, C., *J. Biophys. Biochem. Cytol.*, **1**, 203 (1955).

[9] Yang, J. T., and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

[10] Jirgensons, B., *Arch. Biochem. Biophys.*, **79**, 70 (1958).

[11] Perlmann, G. E., *Arch. Biochem. Biophys.*, **65**, 210 (1956).

[12] Blumenfeld, O. O., J. Léonis, and G. E. Perlmann, to be published.

[13] Schellman, J. A., *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **30**, Nos. 21–27 (1958).

[14] Harrington, W. F., *Nature*, **181**, 997 (1958).

[15] Van Vunakis H., and R. M. Herriott, *Biochim. Biophys. Acta*, **23**, 600, 1957.

[16] Blumenfeld, O. O., and G. E. Perlmann, *J. Gen. Phys.*, **42**, 563 (1959).

[17] Blout, E. R., and R. H. Karlson, *J. Am. Chem. Soc.*, **80**, 1259 (1958).

[18] Fassman, G. D., *Abstr. 4th Intern. Congress of Biochemistry*, Vienna, 1958.

[19] Harrington, W. F., and M. Sela, *Biochim. Biophys. Acta*, **27**, 24 (1958); *Abstr., 133rd Meeting, Am. Chem. Soc.*, San Francisco, 1958.

[20] Kurtz, J., A. Berger, and E. Katchalski, *Nature*, **178**, 1066 (1956).

---

## *UNITY IN THE SPECIFICITY OF ENZYME AND ANTIBODY INDUCTION BY THE SAME DETERMINANT GROUPS**

BY STUART W. TANENBAUM, MICHAEL MAGE,† AND SAM M. BEISER

DEPARTMENT OF MICROBIOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY

*Communicated by E. L. Tatum, May 13, 1959*

*Introduction.*—For many years it has been hypothesized that induced enzyme formation and antibody production are closely related processes. As pointed out by Pollock,[1] this proposal was made by Dienert in 1900, who described the "adaptation" of yeast to galactose, at the time when immunochemical phenomena were first coming under intensive investigation. A wealth of experimental evidence has since accumulated, showing that both of these protein-forming systems utilize amino acids rather than larger peptide units as precursors, that they are under strict genetic control, and that the site of guidance for their specificity may be a template of ribonucleoprotein. The extensive literature which has led to these conclusions has been reviewed by Borsook,[2] Spiegelman,[3] Haurowitz,[4] and Burnet.[5]

One major difference in the detailed mechanisms involved in induced enzyme *versus* antibody protein formation lies in the well-known fact that the former process is initiated by an inducer (determinant) of low molecular weight and simple structure, whereas the latter system requires an antigen having an obligatory molecular