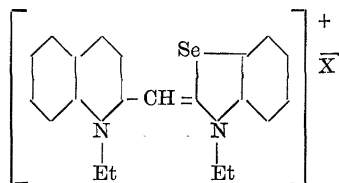


Z-band on excitation by light. The particular electron distribution in water molecules⁷ is believed to lend itself to this and other participation of "hydrate" water in light absorption.

From the necessary alternation of "inactive" and "active" cells, the band intensity will grow with length of filament, but the characteristic frequency or wavelength will not change. Thus the structure behaves like the *meta*-bonded rather than the *para*-bonded polyphenyl.⁸ Symbolizing such a cross-resonance by $N_p \times N_q$ the frequency, intensity and purity (sharpness) of the Z-band will depend upon p and q , hence on the actual structure of the component molecules, because the *basicity* of the related N-atoms is determinative.⁹ For example, the dye *di-ethyl-ψ-selenacyanine* was found by Scheibe⁵ to give a Z-band. But



compared with that of the *symmetrical* ψ-cyanine it was relatively weak and diffuse and at shorter wavelength. This is predicated on the structure proposed. The non-symmetrical ψ-cyanine, when ordered in a Z-state, can give, according to the apposition of the molecules in the cells, *three* intermolecular bands, *viz.*, $N_p \times N_q$, $N_{p'} \times N_{p'}$, and $N_q \times N_q$. Hence the observed band is relatively broad and weak. Lower intensity can be predicated also with asymmetric dyes because parallel paired identical intermolecular transitions are not possible. This condition, as shown by R. S. Mulliken¹⁰ in molecular spectra (*e.g.*, H_2 molecule) makes for great intensification. On the structure proposed, a mixture of two dissimilar but pairable dyes can give, in addition to their own iso-molecular cells or doublets, hybrid doublets and two new related Z-bands, *e.g.*, with ψ-cyanine and the ψ-selenacyanine, $N_p \times N_{p'}$ and $N_p \times N_q$. This is the interpretation of Scheibe's important observation with these two dyes; he found with mixtures a series of intermediate bands which could not be compounded by simple superposition of the 100 per cent. bands. Actually, his data show definite breaks in the neighborhood of 1:1 molecular composition, but such breaks might occur at other proportions, depending upon the degree of hybrid doublet formation in the filaments.

Scheibe has objected to Jelley's "nematic phase" from

evidence that the Z-state occurs independently of the anion. However, not only has Jelley⁶ obtained definite evidence of anion influence, but our conductivity data indicate disappearance of anions in the Z-state. The structure now proposed indicates a new type of nematic phase, constituted of plurimolecular filaments instead of elongated molecules. Similar mesomorphic phases may occur with other dyes than the cyanines, *e.g.*, with the porphyrins and phthalocyanines. With these, however, intermolecular hydrogen bridges bonding key atoms seem more probable, with different conditions for intermolecular resonance.

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ASSOCIATION OF PHOSPHATASE WITH A MATERIAL IN KIDNEY SEDIMENTABLE AT HIGH SPEED AND ITS LIBERATION BY AUTOLYSIS¹

EXTRACTS of many normal and tumor tissues have been shown to contain large amounts of material which can be sedimented in the ultracentrifuge at 27,000 r.p.m. for one hour. The viruses of fowl leukosis and sarcoma^{2,3} substances showing cytochrome oxidase and succinic dehydrogenase⁴ activity, the heterogenetic, tissue and organ specific antigens,^{5,6} have been shown to be associated with this fraction.

The effect of autolysis on tissue extracts was studied in an attempt to obtain information on the relation of the heavy fraction to biologically active constituents of tissues which are of much lower molecular size. Kidney phosphatase was selected because it is usually prepared from autolyzed tissues and is extremely stable in solution.

Kidneys from ten mice were divided into two equal parts. One part (A) was allowed to autolyze at room temperature for a week with 20 volumes of distilled water and a small amount of toluene. The other part (B) was kept frozen at -60° until the first half was autolyzed. It was then ground with sand and 20 volumes of distilled water and toluene. Sodium chloride was added to both solutions to make a final concentration of 0.9 per cent., and the material was centrifuged in the cold and at 8,000 r.p.m. for 15 minutes. These crude extracts were centrifuged at 27,000 r.p.m. during one hour, the supernatants decanted, and the sediments resuspended in the original volume of saline.

These fractions from autolyzed and non-autolyzed

⁷ J. D. Bernal and R. H. Fowler, *Jour. Chem. Phys.*, **1**: 515, 1933.

⁸ Cf. A. E. Gillam and D. H. Hey, *Jour. Chem. Soc.*, p. 1170, 1939.

⁹ L. G. S. Brooker, R. H. Sprague, C. P. Smyth and G. L. Lewis, *Jour. Am. Chem. Soc.*, **62**: 1116, 1940.

¹⁰ R. S. Mulliken, *Jour. Chem. Phys.*, **7**: 32, 1939.

¹ This investigation was supported by grants from the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research.

² E. A. Kabat and J. Furth, *Jour. Exp. Med.*, **71**: 55, 1940.

³ A. Claude, *SCIENCE*, **90**: 213, 1939; **91**: 77, 1940.

⁴ K. G. Stern, Cold Spring Harbor Symposia on Quantitative Biology, **7**: 312, 1939.

⁵ J. Furth and E. A. Kabat, *SCIENCE*, **91**: 483, 1940.

⁶ W. Henle and L. A. Chambers, *SCIENCE*, **92**: 313, 1940.

tissue were analyzed for nitrogen, complement fixing power to tissue specific (Ts) and heterogenetic antibodies (F), and phosphatase activity. The latter was determined under optimal amino acid and magnesium concentration, as described by O. Bodansky,⁷ at several slightly different pH to obtain the maximum activity. Phosphatase activity $Q_{0.05}$ is the reciprocal of the time in minutes necessary to liberate 0.05 mg P per ml of solution. The phosphatase activity of each crude extract was arbitrarily considered as 100 per cent.

this large particle would provide a structural form within the cell which may influence the sequence and direction of enzyme reactions.⁸

Summary: Phosphatase in extracts of mouse kidney is associated with the material sedimentable at 27,000 r.p.m. for one hour. On autolysis, this fraction decreases in amount, phosphatase is liberated, and the tissue specific and heterogenetic components are destroyed.

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TABLE 1

	(A) Kidney autolyzed for 1 week				(B) Kidney ground with sand in cold					
	N/ml	Highest dilution giving complement fixation		Phosphatase activity		N/ml	Highest dilution giving complement fixation		Phosphatase activity	
		Ts	F	Q _{0.05}	%		Ts	F	Q _{0.05}	%
Experiment 1	(2.16 gm kidney used)									
Crude extract	0.71	0	0	.0526	100	0.79	1/5	1/5	.0167	100
27000 supernatant		0	0	.0526	100		0	0	.0072	46
% high speed sediment	2.8 per cent.					15.8 per cent.				
Experiment 2	(1.11 gm kidney used)									
Crude extract	0.60	0	0	.074	100	0.86	1/9	1/3	.0222	100
27000 supernatant		0	0	.074	100		0	0	.0052	26
Resuspended high speed sediment		0	0	0	0		1/9	1/4.5	.0164	82
% high speed sediment	3.3 per cent.					12.0 per cent.				
Experiment 3	(3.3 gm kidney used)									
Crude extract	0.69	1/9	0	.0394	100	0.77				
27000 supernatant		0	0	.0358	91		1/27	1/3	.0312	100
Resuspended high speed sediment		1/9	0	.0078	20		0	0	.0036	12
% high speed sediment	4.4 per cent.					13.7 per cent.	1/27	1/3	.0264	85

Table 1 shows that in tissue extracts (B), the phosphatase activity is sedimented with the tissue specific and heterogenetic antigens. Autolysis of the tissue (A), however, causes a decrease in the amount of the heavy fraction, loss of complement fixing power to both the tissue specific and the heterogenetic antigens, with the liberation of the phosphatase in an active non-sedimentable form. Loss of complement fixing power and liberation of phosphatase from the heavy fraction seem to parallel each other. Thus in experiment 3A some tissue specific antigen remained after autolysis and a small amount of sedimentation of the phosphatase activity was found. Since some autolysis occurs even in the cold, it is not surprising that all phosphatase can not be sedimented in the non-autolyzed extracts (B). When the procedure is carried out more rapidly (within 6 hours) (experiments 2B and 3B), a much larger part of the phosphatase sediments than if carried out more slowly (over 2 days) (experiment 1B).

The association of enzymes of low molecular weight, such as phosphatase, with particles several hundred times larger in size, suggests that this heavy fraction may play an important role in the cell by acting as a carrier for certain biologically active substances. Attachment of different enzymes at various sites on

THE INADEQUACY OF SYNTHETIC DIETS FOR MICE

DURING an investigation on vitamin E and experimental tumors,¹ it was found that strain A males (Bar Harbor) developed edema and protrusion of the eyeballs on a diet supposedly adequate, save for vitamin E. The basal diet (C) consisted of casein 31, corn starch 28, lard 21, salt mixture 7, cod liver oil 3 and yeast 10. Two years ago experiments were started to determine, if possible, the cause of the edema and eye condition. In the first experiment, strain A males, 4 to 5 weeks old, were placed upon diet C and divided into three groups. One group received the basal diet alone, one group received the basal diet plus the oral administration of .5 mg ascorbic acid (Eastman) three times weekly, and the final group received 30 mg of a tested vitamin E concentrate every two weeks. Ten mgs of this E concentrate would insure pregnancy in E deficient strain A females. The growth and physical appearance of the mice were excellent until the mice reached the age of 8-12 months. At this time the following symptoms occurred: sore eyes, uni- or bilateral, characterized by swelling and inflammation of the eyelids leading to closure of the eyes and blindness

⁸ I. M. Korr, Cold Spring Harbor Symposia on Quantitative Biology, 7: 74, 1939.

¹ C. Carruthers, *Am. Jour. Cancer*, 35: 546.

⁷ O. Bodansky, *Jour. Biol. Chem.*, 118: 341, 1937.