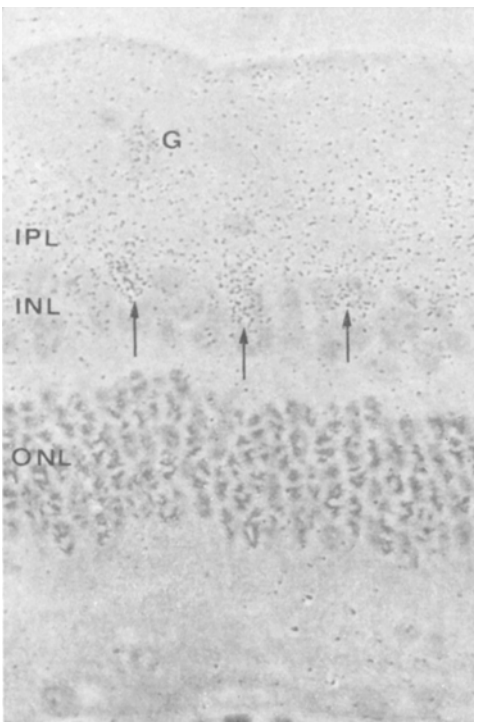
because it can very easily be exposed to exogenous substances by injecting these intravitreally.

GABA has been demonstrated in the dog, ox and rabbit retina[3,4], as has the enzyme synthesizing GABA[4]. Moreover, GABA has been shown to influence retinal nervous mechanisms[5–7].

Tritiated GABA (10–25 μC) was injected intravitreally into rabbit eyes. After 4 h, the retina was dissected out, frozen in a liquid propane-propylene mixture cooled by liquid nitrogen, freeze-dried, fixed with dry gaseous formaldehyde, embedded in vacuo directly in Durcopan ACM (Fluka), sectioned in an LKB Pyramitome, and covered with autoradiographic stripping film (Kodak AR 10). As judged by the excellently preserved monoamine fluorescence, which is highly sensitive to water, diffusion is negligible with this procedure.

The radioactivity of the retina (Figure) was mainly localized in the inner plexiform layer. No sublayering has yet been demonstrated. There was also some radioactivity in the innermost part of the inner nuclear layer and in the ganglion cell and nerve fibre layers. In addition, some cells occupying the same position as amacrine cells were radioactive, as were some nerve cells of the ganglion cell layer.



Autoradiogram, rabbit retina, intraocular injection of 25 μC GABA, 4 h. Radioactivity is seen over a ganglion cell (G) and over cells in the innermost part of the inner nuclear layer (arrows). There is also diffuse radioactivity in the inner plexiform layer (IPL), the ganglion cell layer, and the nerve fibre layer. × 700.

It has been shown that most of the GABA taken up into brain slices is retained as such[8]. Also, the distribution of exogenous and endogenous GABA in rat cerebral cortex is very similar in differential and gradient centrifugal fractions[9]. There is thus little reason to suppose that GABA metabolites interfere to any considerable extent, and, presumably, the radioactivity signifies GABA-containing neurons. This is further supported by the fact that the endogenous GABA of the rabbit retina has been found mainly in parts containing the ganglion cell layer and the inner plexiform layer[4] which agrees well with the present results.

It is of interest to note that there is now evidence suggesting 4 different transmitter substances in the inner nuclear – inner plexiform regions of retina: GABA (as judged from the present work), acetylcholine (as judged from acetylcholinesterase studies[10]), dopamine, and noradrenaline (as judged from fluorescence microscopy[11–13]).

Résumé. En employant la technique autoradiographique, on constate que l'acide γ-aminobutyrique est accumulé principalement dans la couche plexiforme interne et dans certaines cellules de la couche de cellules ganglionnaires et de la couche de cellules amacrines.

B. EHINGER

Departments of Experimental Ophthalmology and Histology, University of Lund, S-22362 Lund (Sweden),
8 April 1970.

[3] K. KOIJMA, K. MIZUNO and M. MIYAZAKI, Nature *181*, 1200 (1958).
[4] K. KURIYAMA, B. SISKEN, B. HABER and E. ROBERTS, Brain Res. *9*, 165 (1968).
[5] W. K. NOELL, Am. J. Ophthal. *48*, 347 (1959).
[6] N. W. SCHOLES and E. ROBERTS, Biochem. Pharmac. *13*, 1319 (1964).
[7] M. STRASCHILL and J. PERWEIN, Pflügers Arch. ges. Physiol. *312*, 45 (1969).
[8] L. L. IVERSEN and M. J. NEAL, J. Neurochem. *15*, 1141 (1968).
[9] L. L. IVERSEN and M. J. NEAL, Br. J. Pharmac. *36*, 206P (1969).
[10] C. W. NICHOLS and G. B. KOELLE, Comp. Neurol. *133*, 1 (1968).
[11] B. EHINGER and B. FALCK, Albrecht v. Graefes Arch. Ophthal. *178*, 295 (1969).
[12] This study was supported by grants from the Faculty of Medicine, University of Lund and was carried out within a research group sponsored by the Swedish Medical Research Council (projects No. B70-14X-712-05 and B70-14X-2321-03).
[13] With a similar technique, HÖKFELT and LJUNGDAHL (personal communication 1970) have recently demonstrated uptake of tritiated GABA in cells in rat cerebellar cortex.

'Rupture Sites' in Elastin During the Course of Organo-Alkaline Hydrolysis

We have studied the various rupture sites of the polypeptide chains produced during the organo-alkaline hydrolysis of elastin by labelling with dinitro-fluorobenzen (DNFB) according to the method of SANGER[1]. We have found 6 sites having identified dinitro-protein-(DNP)-aspartic acid, DNP-glutamic acid, DNP-glycine, DNP-alanine, DNP-valine and DNP-leucine[2].

The distinction between DNP-glutamic acid and DNP-serine is sometimes doubtful. To obtain more rigorous separations, we carried out a series of two dimensional chromatograms of the organo-alkaline effected elastin lysate labelled with DNFB (elastin Kp[2]) using the following solvents: 1. Toluene/Pyridine/2-chloroethanol/0.8 *N* Ammonium hydroxide (100:30:60:60). 2. Chloroform/Benzyl alcohol/Acetic acid (70:30:3). We were able to observe 4 poorly separated spots of Rf lower than that of DNP-glycine in the 2 solvent systems used (Figure 1). These spots were eluted with ether and

rechromatographied. However, satisfactory results were not obtained because this method is not sensitive enough to detect amino acids of such low concentration, thus leaving their identification unresolved.

Using dimethyl-amino-naphtalene-5-sulfonyl or Dansyl chloride[3] to label N-terminal amino acids, it was possible to detect residues at concentrations of less than 1% of the total input residues. Indeed, on a preparation of elastin Kp, the number of rupture sites identified was greater than that found previously, and moreover, rather representative of the amino acid composition of fibrous elastin established after hydrolysis in $6N$ hydrochloric acid (under nitrogen) for 72 h (Figure 2). The solvents used here were: 1. Benzene/Pyridine/Acetic acid (80:20:5). 2. Toluene/monochloroethanol/25% Ammonium hydroxide (6:10:4). We were able to identify dimethyl-amino-naphtalen-5-sulfonyl-(DNS)-aspartic acid, DNS-glutamic acid, DNS-serine and DNS-threonine. This last is particularly interesting: PARTRIDGE[4] found quite a while ago that the first amino acids to be liberated during the course of acid hydrolysis were aspartic and glutamic acids only. Likewise, at the N-terminals, we have identified DNS-proline, DNS-phenylalanine as well as a spot corresponding to DNS-dilysine. Moreover, thanks to this technique, we have been able to distinguish between DNS-leucine and DNS-isoleucine. The spots of Rf greater than that of isoleucine indicate peptides containing isoleucine in the N-terminal position resistant to at least 48 h hydrolysis, according to KELLER and MANDL[5].

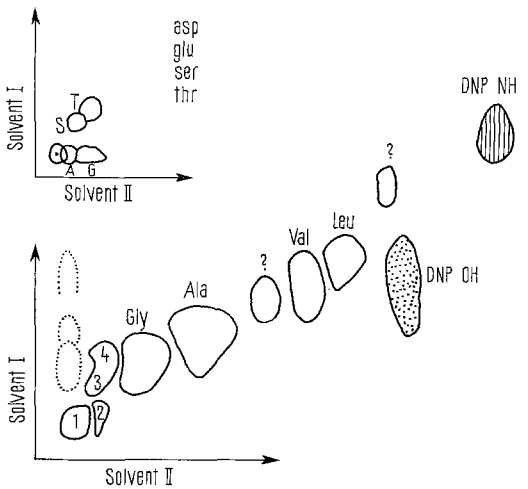


Fig. 1. Two dimensional silica-gel (Merck) thin layer chromatography of dinitrobenzene labelled elastin (Ether soluble DNP-amino acids). The labelling was done according to the method of SANGER[1]. Solvent I: Toluene/Pyridine/monochloro ethanol/0.8N ammonium hydroxide (100:30:60:60) (v/v). Solvent II: chloroform/benzyl alcohol/acetic acid (70:30:3) (v/v).

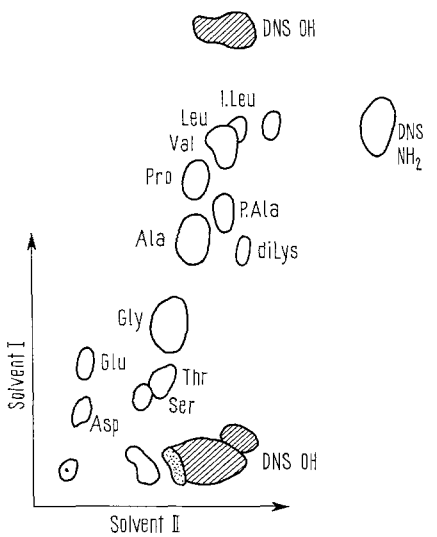


Fig. 2. Two dimensional silica-gel (Merck) thin layer chromatography of 'dansylated' elastin Kp. The dansylation was done according to the method of Gray and Hartley. Solvent I: benzene/pyridine/acetic acid (80:25:5) (v/v). Solvent II: Toluene/monochloro-ethanol/ammonium hydroxide 25% (6:10:4) (v/v).

The problem concerning the contigent dilysine is more complex because it is a question of knowing whether this peptide comes from an unknown bridge bond or possibly metabolic intermediate of desmosine or isodesmosine.

In short, by comparing the results obtained by the 2 methods we may conclude that there is no special rupture site on the polypeptide chains. The alkaline hydrolysis effected under our conditions in the presence of alcohol is responsible for a random proteolytic degradation due to a simple hydrolysis in which the organic constituent plays the role of an 'accelerator' in opening regions closed off by the hydrophobic amino acid residues, thereby favoring the role of the hydrolytic agent ($1M$ potassium hydroxide) which can then render the peptide bonds broken, but with no specificity.

We have other evidence to demonstrate such cutting. During the course of progressive hydrolysis of fibrous elastin to elastin Kp by $0.6M$ potassium hydroxide (sufficient to disperse elastin) in 80% ethanol, we have carried out the following analyses: 1. Comparison of amino acids as a function of time of hydrolysis. 2. Approximate measure of molecular weight by passage through biogel columns (Biorad).

From the results obtained, it appears that from the first hour of hydrolysis onward, the amino acid composition of the peptides obtained is very similar to that of fibrous elastin, with the exception of the lysine, desmosine, isodesmosine group. The measurement of relative molecular weights carried out in the biogel columns gave us numbers varying from 2000 to 4000 daltons, according to the time of hydrolysis. This latter makes quite apparent to us the drastic effect of the potassium hydroxide during this hydrolysis.

Even though the 'accelerating' role of the alcohols is clear, from the facts concerning their action on the tertiary structure, the rupture of the peptide chain appears to be completely random.

Résumé. Au cours de l'étude des peptides résultant de la scission de l'élastine par hydrolyse en milieu organo-alcalin, nous avons été obligés de préciser certains points du marquage. En utilisant la méthode au DNS, nous en avons déduit que l'hydrolyse dans nos conditions, même en présence de solvants, s'effectue d'un manière non systématisée.

N. KORNFELD and P. REBEYROTTE

Institut d'Immuno-Biologie de l'I.N.S.E.R.M., Hôpital Broussais, 96, rue Didot, Paris 14e (France), 7 April 1970.

[1] F. SANGER, Biochemical J. *40*, 261 (1946).
[2] N. KORNFELD-POULLAIN, P. REBEYROTTE and M. TURPIN, Bull. Soc. Chim. biol. *52*, 3 (1970).
[3] C. GROS and B. LABOUESSE, Eur. J. Biochem. *7*, 463 (1969).
[4] S. M. PARTRIDGE, M. F. DAVIS and G. S. ADAIR, Biochem. J. *61*, 11 (1955).
[5] S. KELLER and I. MANDL, Biochim. biophys. Research Comm. *35*, 687 (1969).