An Effect of Pyridoxal-5′-Phosphate on the Strength of Skin in vitro

A number of compounds containing thiol groups with an amino group on an adjacent carbon atom have been found to reduce the strength of collagenous tissues when applied directly to them in vitro (skin[1,2]; tendon[3]). One such compound (penicillamine) has the same effect in vivo[4].

It has been suggested that these effects are brought about by action of the reagents on intermolecular links, in collagen, formed by the condensation of an aldehyde and amino group to make an azomethine bond[5,3]. Recently direct chemical evidence of the presence of such a link in native collagen precipitated from solution has been obtained[6]. Evidence regarding the function of such a link in mechanical properties of tissues is, however, less clear. The activity of penicillamine and other amino thiols appears to be connected with their ability to react with aldehyde groups. No corresponding effects of an amino group reacting substance on mechanical properties of collagenous tissue appear to have been described. We have examined the effect of pyridoxal-5′-phosphate (Pxl-P).

Materials and methods. Pairs of rings of skin from the tail of rats (males, 80–150 g weight, 1–2 months old) were loaded separately to breaking point as described previously[7]. Two rings were broken, untreated, immediately after the animal was killed and the strength of treated rings is expressed as a percentage of the value obtained.

Rings were treated in pairs in 2 ml of solution either in the refrigerator (at 4°C about) or incubated at 37°C. Reagents were made up in physiological saline buffered with phosphate (NaCl-P, consisting of NaCl, 0.1 *M*, Na_2HPO_4 0.021 *M* adjusted to required pH with HCl or NaOH). Pxl-P solutions were kept in the dark. The pH of solutions was measured at the end of treatment with a glass electrode.

Reagents were obtained commercially (Koch-Light; Pxl-P, pyridoxal HCl, pyridoximine di-HCl, hydroxylamine HCl; British Drug Houses, Pxl-P, hydrazine SO_4, $NaBH_4$; Roche, lysine HCl). The purity of one sample of Pxl-P was checked by measurements of its spectral absorption curve which were found to correspond with measurements in the literature[8,9].

Results. Effect of Pxl-P. Treatment with Pxl-P (10 m*M*) was found to reduce the strength of the skin progressively (Figures 1 and 2).

The effect was largely reversible, strength returning when rings were returned to simple buffered saline (Figures 1 and 2). The return seems to be not quite complete, possibly because an effect of treatment is usually to detach the epidermis, as a separate intact layer, which must involve breakdown of at least some structural material.

Specificity of effect. Treatment with pyridoxamine or pyridoxine (10 m*M*, 4°C) had no comparable effect (Figure 1). The effect of Pxl-P was reduced in the presence of lysine (Figure 1).

Inhibition of effect. Pretreatment with $NaBH_4$ (0.1 *M*, Ph 10.5, 1 h, 20°C) followed by washing in NaCl-P at pH 7.5, largely inhibited the effect of Pxl-P. Mean strength of 5 pairs of rings treated first with $NaBH_4$ (0.1 *M*, 1 h, 20°C) followed by Pxl-P (10 m*M* in NaCl-P, pH 7.5, 4°C, 1–3 days) was 188 ± 7 (% untreated), followed by NaCl-P alone 215 ± 10.

Pretreatment with NH_2OH (hydrochloride, 0.1 *M*, $1^1/_2$ h, pH 9, 20°C) followed by washing in NaCl-P at pH 7.5 also inhibited the effect of Pxl-P. Figures for

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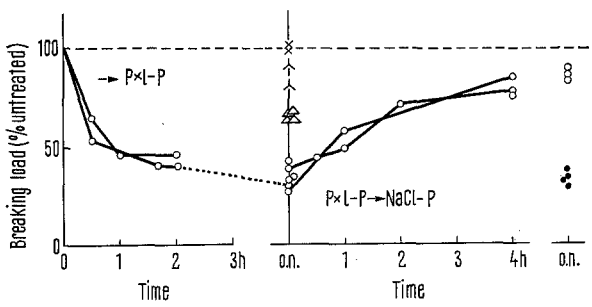

Fig. 1. Effect of pyridoxal-5′-phosphate on strength of skin. This figure shows the results of a number of experiments; each point is the mean of a pair of rings of skin; lines join means of pairs, all from one tail. Rings of tail skin were either incubated at 37°C, in phosphate-buffered physiological saline, pH 7.4, containing 10 m*M* Pxl-P (time scale hours on left); or left overnight (o.n.) in the same solution at 4°C. Results for rings treated with pyridoxine (×), or pyridoxamine (λ, o.n., 4°C, 10 m*M* in NaCl-P, pH 7.4), or with Pxl-P in presence of 10 m*M* lysine (△) are also shown; also results for rings treated for 1 h in 0.1 *M* hydroxylamine (pH 9, 20°C, ●) after Pxl-P and before transference back to physiological saline (○).

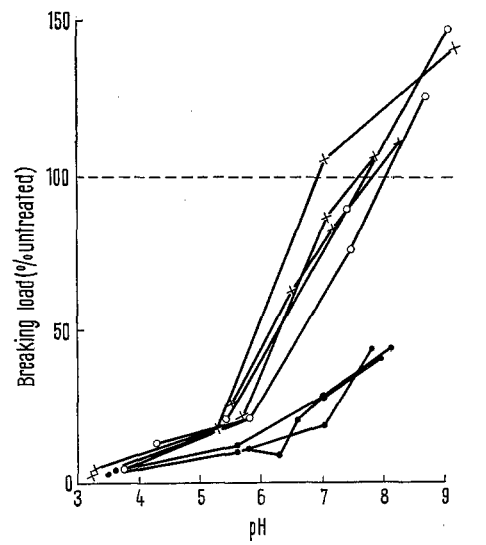

Fig. 2. The effect of pyridoxal-5′-phosphate at different hydrogen ion concentrations. Skin was treated with 10 m*M* Pxl-P in phosphate buffered physiological saline at various pH levels (overnight 4°C, ●), or in buffered saline alone (×). The effect of this treatment followed by return to physiological saline alone is also shown (○). Lines join means of pairs from a single tail.

strength, in 3 successive experiments with increasing length of Pxl-P treatment, were: hydroxylamine pretreated, 76, 70; 88, 82; 75, 64; NaCl-P pretreated, 52, 45; 35, 33; 29, 20.

Inhibition of reversal. Treatment with NH_2OH (hydrochloride 0.1 *M*, pH 9, 1 h, 20°C) after Pxl-P prevented recovery of strength on subsequent transfer to NaCl-P alone at pH 7.5 (Figure 1); NH_2NH_2 (sulphate) had a similar effect.

Discussion. Pxl-P is a co-enzyme in a number of reactions involving amino-groups (see review by BANKS[10]) and is known to react with them. The hypothesis that the effect of Pxl-P on strength is brought about by rupture of azomethine linkages formed between an aldehyde and amino-group would explain the inhibition of recovery of strength by NH_2OH and NH_2NH_2. It would also provide a reasonable explanation of the stabilizing effect of previous reduction with $NaBH_4$. It is, however, not clear why pretreatment with NH_2OH or NH_2NH_2, acting on a link of this nature, should have a stabilizing effect. BENSUSAN, MCKNIGHT and NAIDU[11], in contrast, report presumptive evidence of the disruption of such a link by NH_2NH_2 in a model compound (furfural-butylamine). The observation that NH_2OH and NH_2NH_2 have this effect is, however, of interest because they also protect skin against the weakening produced by lowered pH, as well as preventing recovery on return to neutral pH[12]. $NaBH_4$ reduction also has a stabilizing effect against pH change, down to about 5[13]. These similarities between effects of Pxl-P and reduction of pH are compatible with the hypothesis that the same links are concerned.

Discussion has been confined to possible explanation of the results in terms of a single effect on collagen. This is likely to be an oversimplification: for example, it does not readily explain the detachment of the epidermis produced by Pxl-P.

The fact that Pxl-P is a naturally occurring substance present in tissues perhaps adds interest to these observations, though normal concentrations in whole tissue are much lower than we used (e.g.[14]). Other naturally occurring substances with a similar effect are cysteine, glutathione[1] and homocysteine[15] (but not reported in detail).

Résumé. Le traitement de la peau de la queue de jeunes rats avec du pyridoxal-5'-phosphate a réduit sa résistance (jusqu'à 70%). Cet effet est en grande partie réversible, la réversibilité pouvant toutefois être inhibée par l'hydrazine ou l'hydroxylamine: ces observations sont en accord avec l'hypothèse attribuant, pour une grande partie, la résistance de ce tissu à des liaisons azométhines, formées par la condensation de fonctions aldéhyde et amine.

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Changes in Electrical Activity of the Cockroach *Blatta orientalis* L. Brain Induced by Anti-Lobster Brain Antibody

We demonstrated in mammals that the electrical activity of the brain and behaviour might be affected by anti-brain antibodies injected into the cerebral cavity[1]. The enormous structural and functional complexity of the mammalian brain compels immunoneurological strategy to include some 'simple systems' in the study. It was considered, therefore, that the insect's brain, because of its relatively simple organization[2], represents a suitable model for the investigations of the biological properties of the anti-brain antibodies.

Materials and methods. One part of homogenized brain from lobster (*Palinurus vulgaris*) was mixed with 2 parts of complete Freund's adjuvant and injected s.c. into rabbits (0.8 ml of antigen-adjuvant mixture per animal). 20–30 days later each rabbit received i.p. 50 mg of lobster brain without adjuvant. The animals were bled at various time intervals, and the sera examined for the presence of anti-lobster brain antibodies by means of different precipitin reactions and complement fixation technique. Sera containing antibodies were pooled, immune γ-globulin fraction isolated[3], dialyzed against physiological solution for insects[4], and lyophilized. Normal rabbit γ-globulin was separated in an identical manner. Prior to use, normal and immune γ-globulins were dissolved separately in distilled water to a concentration of 40 mg/ml, and then 12 50% hemolytic units of guinea-pig complement were added to each solution.

The experiment was carried out on adult cockroaches (*Blatta orientalis* L.). After anaesthesia in carbon dioxide, the chitin cover from the frontal part of the head was removed, and steel or Ni-chrome bipolar and monopolar electrodes, having a tip of approximately 50 µ, were implanted in the brain as described previously[5]. In most instances the electrodes were inserted into the protocerebrum (Figure 1). Physiological solution, normal γ-globulin and immune γ-globulin were applied on the surface of the insect's brain with a glass micro-pipette. The electrical activity was recorded for at least 3 h using an 'Alvar' encephalograph.

Results and discussion. In spite of the wide phylogenic distance between the lobster and the rabbit, 6 of 15 rabbits injected with the lobster brain in adjuvant devel-

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