Quantitative Researches on the Optical Properties of Human Bone

THE quantitative studies of Dallemagne and Mélon[1] on the optical properties of ox bone (already described in part in *Nature*[2]), and their deductions concerning the relations between the organic and the inorganic fractions of the bone, have led me to a series of analogous, independent researches on human bone.

In specimens from subjects three to sixty years old, the index of refraction at room temperature of air-dried human bone (femoral diaphysis) was found to be almost constant and ranged around $1 \cdot 560 \pm 0 \cdot 002$. From birth until the age of three years, the index of refraction appears to rise at an increasing rate from $1 \cdot 549$ to $1 \cdot 560$. In the seventh and eighth decades, there is a small but noticeable increase : $1 \cdot 564 \pm 0 \cdot 002$. Bone from which the organic fraction has been removed shows a particularly interesting behaviour which to my knowledge has not been pointed out before : the index of refraction estimated by the methods of Becke and Schröder van der Kolke varies in value according to the nature of the comparison fluids. A value of $1 \cdot 600 \pm 0 \cdot 002$, almost constant for all ages, is obtained by using a mixture of oil of lavender and oil of cinnamon, which in all probability gives results more closely approximating the real values. Technical difficulties do not allow precise measurements of the index of refraction of fresh bone or of bone deprived of its mineral fraction. However, it is probable that the index of refraction of fresh bone from adult subjects is not less than $1 \cdot 540$.

The birefringent power of normal human bone ranges about $+ 0 \cdot 004$, as determined from measurements on seven subjects of 1, 3, 7, 11, 25, 50 and 80 years of age.

The quantitative researches clearly agree with the fact, already observed qualitatively by Schmidt[3], that normal human bone, deprived of its organic fraction, follows Wiener's law[4] of birefringence of form (*Formdoppelbrechung*). The parabolic curve of birefringence of form has a negative vertex. It follows from this that the mineral fraction of the bone is anisotropic and has its own negative birefringence (*Eigendoppelbrechung*). With due reservations on the more or less perfect penetration of the fluids in bone deprived of its organic fraction, the birefringent power of the mineral fraction is not constant, but varies with the age, tending to diminish rapidly in the first years of life, while remaining practically stationary during adult and old age.

The conclusions which Dallemagne and Mélon[1] wish to draw from their researches on the optical properties of ox bone, namely, "La fraction organique de l'os, représentée par l'osseine, n'accuse . . . aucun lien chimique perceptible aux méthodes physiques avec le phosphate tricalcique, élément principal de la fraction minérale", do not seem to me acceptable without reserve, and this for two fundamental reasons : (1) A chance coincidence may be responsible for the identical values obtained by these authors for the total birefringence measured directly and for the indirectly calculated birefringence. Indeed, the index of refraction and the relative volume of the organic substance measured by Dallemagne and Mélon do not apply only to the fundamental interfascicular substance (*Kittsubstanz*) to which the mineral fraction is bound, but rather to the complex mass consisting of fundamental substance + collagen fibres.

(2) Dallemagne and Mélon also do not take into consideration the birefringence of the organic substance (fundamental substance + fibres), which certainly has an influence on the value of the total birefringent power of the bone.

ASCENZI ANTONIO

Centro di Studio per la
Fisio-Patologia,
Consiglio Nazionale delle Ricerche,
Istituto di Patologia Generale,
University of Rome.

[1] Dallemagne, M. J., and Mélon, J., *Bull. Soc. Roy. Sci., Liége*, **12**, 558 607 (1943) ; **13**, 254 (1944) ; *Bull. Soc. Chim. Biol.*, **27**, 85 (1945) *J. Washington Acad. Sci.*, **38**, 181 (1946).
[2] "Biochemistry in Belgium", *Nature*, **159**, 617 (1947).
[3] Schmidt, W. J., *Ber. Oberhess. Ges. Natur- u. Heilk.*, **15** (1932–33) *Naturw. Abt. Giessen*, 219 (1933).
[4] Wiener, O., *Abt. sächs. Ges. Wiss., math. phys. Kl.*, **32**, 509 (1912).

Synthesis of γ-Glutamyl Peptides

IN the classical synthesis by Bergmann and Zervas[1], carbobenzoxy L-glutamic anhydride reacts with amino-acid esters to yield, after saponification and hydrogenation, α-glutamyl peptides. There have, however, been indications that the anhydride ring may not open exclusively in this direction. Bergmann[2] reported that glycylglycine ethyl ester yields a mixture of the α- and γ-derivatives, and in the analogous reaction with ammonia Melville[3] found some 14 per cent glutamine admixed with the *iso*-glutamine. Anomalies have also been observed in the opening of the ring of carbobenzoxy L-aspartic anhydride[4].

In the course of an investigation of ring openings of this type, we have examined the reaction of carbobenzoxy L-glutamic anhydride with cold aqueous hydrazine. The crystalline product, isolated in nearly quantitative yield (m.p. 170–171°), was found to be a mixture of the α- and γ-hydrazides, which could not be separated by repeated crystallization. Conversion to the azide and coupling with glycine ethyl ester gave a product from which carbobenzoxy α-L-glutamyl glycine could be isolated in low yield, but which on saponification and hydrogenation gave a mixture of α- and γ-peptides, since with ninhydrin $0 \cdot 8$ moles of carbon dioxide were evolved.

We were therefore led to prepare pure carbobenzoxy γ-L-glutamyl hydrazide by the action of hydrazine on the carbobenzoxy γ-ethyl-L-glutamate of Abderhalden and Nienburg[5]. This hydrazide, which clearly should provide an unambiguous path for the synthesis of γ-glutamyl peptides, has recently been described by Hegedüs[6], who has demonstrated its usefulness in a novel synthesis of glutathione. We record, however, m.p. 178–179° (uncorr.) for our carbobenzoxy γ-L-glutamyl hydrazide, which we obtained in quantitative yield on treating the ester with hydrazine in aqueous ethanol at 20° for 48 hours (found : C, $52 \cdot 7$; H, $5 \cdot 9$; N, $14 \cdot 0$; $C_{13}H_{17}O_5N_3$ requires C, $52 \cdot 86$; H, $5 \cdot 81$; N, $14 \cdot 23$ per cent. $[\alpha]_D^{18} - 13 \cdot 4° \pm 0 \cdot 4°$ in $0 \cdot 5 N$ hydrochloric acid, c, $6 \cdot 4$; $[\alpha]_D^{18} - 5 \cdot 1° \pm 0 \cdot 3°$ in water containing 1 equiv. NaOH, c, $10 \cdot 0$). Hegedüs records m.p. 170–172° (uncorr.), and 44 per cent yield.

Our carbobenzoxy γ-L-glutamyl hydrazide yielded a benzylidene derivative, m.p. 200–201° (found : C, $62 \cdot 5$; H, $5 \cdot 7$; N, $11 \cdot 2$; $C_{20}H_{21}O_5N_3$ requires C, $62 \cdot 64$; H, $5 \cdot 52$; N, $10 \cdot 96$ per cent). Conversion to the azide and coupling with glycine ethyl ester in chloroform solution gave carbobenzoxy γ-L