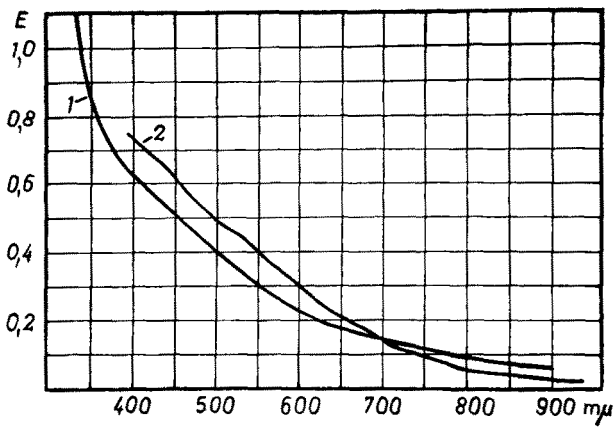
Absorptionsspektrum von Melaninpigment[1]

Melaningranula im Durchmesser von etwa 2–4 μ, von einem Fall von ausgedehnter Melanoblastomatose beim Menschen isoliert und einzeln mikrospektrophotometrisch ausgemessen, zeigen folgendes Extinktionsdiagramm (siehe Abbildung, Kurve 2): die Absorption beginnt im roten Bereich des sichtbaren Lichtes und steigt von dort zunehmend und stetig an bis zum violetten Ende des Spektrums. Ein ausgesprochenes Extinktionsmaximum fehlt.



Extinktionsdiagramme von einzelnen Melaninpigmentkörnchen (Kurve 2) und vom KOH-Auszug einer Melanoblastommetastase (Kurve 1).

Im allgemeinen muss man bei mikrophotometrischen Messungen in diesen Dimensionen mit einem mehr oder weniger stark ausgeprägten Streufehler rechnen, dessen Ausmass bekanntlich zur kurzwelligen Seite des Spektrums hin erheblich zunimmt. Modellversuche an wässerigen Thioninlösungen, deren Extinktionsmaximum bei 610 mμ liegt und sehr scharf begrenzt ist, und an Thioninkristallen von ähnlicher Grössenordnung wie die gemessenen Melaningranula hatten das sehr deutlich gezeigt. Wenn man zur photometrischen Analyse allerdings Pigmentkörnchen auswählt, die möglichst frei von optischen Inhomogenitäten erscheinen und mit hoher numerischer Apertur (1,25) des Mikroskopobjektivs arbeitet, dann lässt sich das so erhaltene Ergebnis sehr gut vergleichen mit dem Extinktionsdiagramm, das *in vitro* – in Küvetten von 1 cm Schichttiefe – im KOH-Auszug vom gleichen Melanoblastom ermittelt wurde (Abbildung, Kurve 1), und ebenso mit den Beobachtungen von EDWARDS und anderen. Es zeigt sich also, dass die rein mikroskopisch erhaltenen Absorptionswerte, die bei den vorliegenden Untersuchungen nach den in [3] angegebenen Verfahren ermittelt wurden, mit den Makrowerten durchaus identisch sind.

J. MEYER-ARENDT und M. BAYER

Pathologisches Institut der Universität Hamburg, den 14. April 1954.

[1] Die Arbeit wurde mit Unterstützung der Deutschen Forschungsgemeinschaft ausgeführt.

[2] E. A. EDWARDS in M. GORDON, *Pigment Cell Growth* (Academic Press Inc., New York 1953), S. 149.

[3] J. MEYER-ARENDT, Beitr. path. Anat. *113*, 388, 1953.

Summary

The microphotometric absorptiometry of single isolated melanin granula and the photometric analysis of a KOH extract from a melanoblastoma metastasis show an extinction strongly increasing to the violet end of the spectrum, without any remarkable extinction maximum.

Infrared Spectrometric Research on the Relation Between Ossein and Inorganic Bone Fraction

Much valuable information remains to be gained on the chemical relations existing between ossein and the inorganic bone fraction.

According to CAGLIOTI[1], the ray pattern of bone gives evidence of a semi-combined lattice resulting from ossein and the inorganic fraction.

The conclusions of DALLEMAGNE and MÉLON[2], that the birefringence of total bone is due to a combination of the optical properties of the organic and inorganic fractions, in accordance with pure physical laws, have been questioned by ASCENZI[3].

Recently, histochemical investigations (RUBIN and HOWARD[4]), studies on the "local factor" of calcification (SOBEL[5]) and radioautographic researches (DZIEWIATKOWSKI[6]) seem to demonstrate that the fixation of calcium in bone is related to sulphuric groups of the ossein. Nevertheless, the nature of the substance containing this groups has not yet been ascertained.

Further, NEUMAN, BOYD, and FELDMAN[7] have produced evidence that, in cartilage calcification, chondroitin sulfate combines with cations and it is semi-saturated with regard to calcium.

On the other hand, POLONOVSKI and CARTIER[8] have found that the first stage of bone calcification corresponds to the phosphorylation of the NH_2 groups of the osteoid. The PO_4 radicals linked in this way to the proteins would also bind calcium.

In this note we report the results of a study by infrared spectrophotometry on total bone, ossein and the inorganic bone fraction.

Experimental.–A Beckman IR_2 spectrometer was used. The samples were prepared from ox femoral diaphysis and they corresponded to (a) sections of total bone 10 micron thick, (b) the same sections decalcified with 2% HNO_3, (c) powder of bone deprived of ossein, according to the method of GABRIEL[9].

[1] V. CAGLIOTI, Atti del Vo Congresso di Chimica Pura ed applicata *1935*, 325.

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[3] A. ASCENZI, Rend. Acc. Naz. Lincei Nota I: Serie VIII *4*, 777 (1948); Nota II: Serie VIII *5*, 100 (1948); Nota III: Serie VIII *5*, 171 (1948); Nature *163*, 604 (1949); Science *112*, 84 (1950). – A. ASCENZI and E. L. BENEDETTI, Arch. Sci. Biol. *38*, 234 (1954).

[4] P. S. RUBIN and J. E. HOWARD, Trans. Macy Conf. Metabolic Interrelations *2*, 155 (1950).

[5] A. E. SOBEL, Trans. Macy Conf. Metabolic Interrelations *4*, 113 (1952).

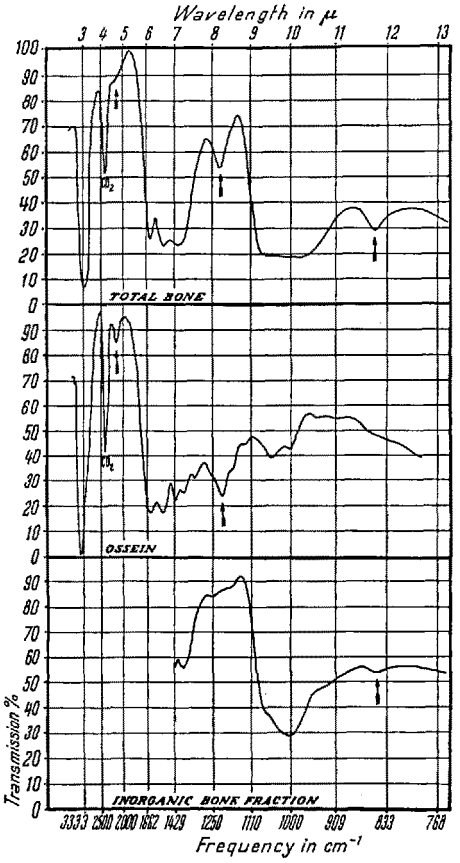
[6] D. D. DZIEWIATKOWSKI, Trans. Macy Conf. Metabolic Interrelations *4*, 74 (1952).

[7] W. F. NEUMAN, E. S. BOYD, and I. FELDMAN, Trans. Macy Conf. Metabolic Interrelations *4*, 100 (1952).

[8] M. POLONOVSKI and P. CARTIER, C. r. Acad. Sci. Paris *232*, 119 (1951).

[9] S. GABRIEL, Hoppe-Seyl. Z. *18*, 257 (1894).

Results.–The infrared spectra of total bone, compared with the spectra of ossein as well as those of the inorganic fraction, showed the following main differences (Figure).



(a) In the ossein spectra an absoption occurs at a frequency of about 2155 cm^{-1}. The same absorption cannot be detected in the spectra of total bone nor in those of the inorganic fraction. Absorption at 1213 cm^{-1}, found in the ossein spectra, decreases in total bone spectra.

(b) An absorption peak at about 850 cm^{-1}, found in the spectra of inorganic bone fraction, appears much increased in the spectra of total bone.

The results of some of the above-mentioned studies, as well as some of our unpublished results on the spectra of very simple substances, suggest that the absorption at 2155 cm^{-1} in the spectra of ossein is the first overtone of the fundamental and totally symmetric frequency at 1213 cm^{-1} related to the $-SO_2-$ grouping[1].

This view is also supported by the work of SCHREIBER[2] on the infrared spectra of many compounds containing the $-SO_2-$ grouping, as well as by the research of MECKE[3] on the SO_2 absorption frequencies.

In this connection the disappearance of the frequency at 2155 cm^{-1} in total bone spectra can easily be explained. In fact it is well known that any modification related to the vibrational energy of a functional grouping induces (a) a decrease of the fundamental absorption band, (b) the disappearance of the first overtone. Both the phenomena are present in total bone and suggest that one or both S–O bonds of the sulfonic groupings pertaining to the ossein may occur in a polar form. Such a condition would increase the electro-negativity of the oxygen which in turn would be able to bind calcium.

The spectra of inorganic bone fraction show the absorption frequencies of the orthophosphates (calcium phosphate, according to CORBRIDGE and LOWE[1], HUNT, WISHERD and BONHAM[2]). A large absorption band with a maximum at 1002 cm^{-1} corresponds to a totally symmetric frequency related to the PO_4 (see also HERZBERG[3]). Another maximum at 850 cm^{-1} strongly increases in total bone. This increase is probably due to a condition of asymmetry related to the PO_4 ion. In this manner the P–O bands would not be energetically equivalent and would determine a change of dipolar moment. The modified phosphoric grouping would form bonds (hydrogen bonds) with the OH or the NH groupings of the fundamental substance of the ossein. The existence of hydrogen bonds is suggested by the displacement observed in the fundamental frequency of the OH grouping (or NH grouping, according to POLONOVSKI and CARTIER[4]). In fact this frequency corresponds to 3546 cm^{-1} in ossein spectra and to 3289 cm^{-1} in spectra of total bone.

Conclusion.–The results of our researches may be summarized as follows. In total bone the bonds between ossein and inorganic fraction occur via the sulfonic groupings of the ossein, the PO_4 groupings of the inorganic fraction and hydrogen bonds.

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Institute of General and Inorganic Chemistry and Institute of Morbid Anatomy, University of Rome, April 14, 1954.

Riassunto

Sulla base di ricerche spettrofotometriche all'infrarosso su sezioni di osso intero, di osso decalcificato (osseina) e di osso privato della frazione organica gli autori sono giunti ai risultati seguenti. Nell'osso intero i legami fra osseina e frazione inorganica sono realizzati dai gruppi solforici dell'osseina, dai gruppi PO_4 della frazione inorganica e da ponti idrogeno.

[1] In the region 1250–950 cm^{-1}, absorption bands are also present related to bonds pertaining to polysaccharides. We have kept account of these bands by means of comparative investigations.

[2] I. C. SCHREIBER, Anal. Chem. *21*, 1168 (1949).

[3] R. MECKE, Z. physic. Chem. B. *16*, 409 (1932).

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[2] J. M. HUNT, M. P. WISHERD, and L. C. BONHAM, Anal. Chem. *22*, 1478 (1950).

[3] G. HERZBERG, *Infrared and Raman Spectra* 5 Ed. (Van Nostrand Co. Inc., New York, 1951).

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Lipoide und Virulenz bei Typhusbakterien

Die Virulenz bestimmter Typhusstämme wird, wie bekannt, einem besonderen, von FELIX[1] entdeckten und von anderen Autoren[2] erforschten Vi-Antigen zugeschrieben. Es interessierte uns, zu prüfen, ob und in welchem Zusammenhange die Virulenz mit dem Lipoidgehalt verschiedener Stämme steht. DENNIS[3] fand nämlich, dass sämtliche Lipoidfraktionen eine ähnliche Wirkung auf verschiedene Gewebe ausüben, wie es sonst unter der Einwirkung von Phtionsäure beobachtet wurde. Wir untersuchten zu diesem Zweck zwei stark virulente

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[3] E. W. DENNIS, Amer. J. Hyg. *32B*, 1 (1940).