lems of our age will emerge. There is much more than an arithmetic increase when two or more disciplines pool their efforts.

If the reader desires to delve more deeply into the mathematical theory of point versus interval estimates for relative risk, he is encouraged to explore the articles by MIETTINEN[13, 14] of the Departments of Epidemiology and Biostatistics, Harvard School of Public Health.

Dr. IRWIN BROSS and members of the biostatistics department at Roswell Park Memorial Institute have been most helpful in providing the necessary scientific atmosphere which has spurred on this new look at the relative risk statistic.

Zusammenfassung.

Seit 25 Jahren hat sich der Begriff «relatives Risiko» bewährt, um die auf Umweltbelastungen zurückgehenden gesundheitlichen Schäden quantitativ zu erfassen. In diesem Beitrag werden die mathematischen Grundlagen dargelegt und die Beziehungen zwischen dem berechneten und dem tatsächlichen Risiko untersucht. Bei kleinem Risiko wird der Fehler geschätzt. Beziehungen zwischen dem relativen Risiko und dem Verhältnis der Häufigkeit aller Erkrankungen zu der Häufigkeit der durch Umweltbelastung hervorgerufenen Erkrankungen werden algebraisch und graphisch dargestellt. Ein dynamisches Populationsmodell erlaubt, die Unterschiede des relativen Risikos innerhalb der Population zu studieren, im Gegensatz zum bisher benützten uniformen Risiko. Dieses Modell ist flexibler und hat sich bei immunologischen Studien als realistischer erwiesen.

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[13] O. S. MIETTINEN, Biometrics *26*, 75 (1970).

[14] O. S. MIETTINEN, Am. J. Epidemiol. *99*, 325 (1974).

SPECIALIA

Detection of Zinc in CHAMPY-MAILLET's Histological Stain by Electron Probe Analysis

MAILLET[1] has proposed the use of an osmium tetroxide zinciodide (ZIO) mixture, to reveal, with some degree of specificity, peripheral nervous structures at histological and cytological levels.

The reducing process of this solution allows simultaneously both fixation and staining of histological preparations: neurites and nerve endings appear stained in black on a yellowish background. The reducing chemical process is still imperfectly known. One of us (J.G.) has suspected that Zinc could also be constitutive of the stain, on account of its amphoteric properties; preliminary chemical tests sustain this view. It seemed therefore interesting to investigate the distribution of Zn, Os and I in stained zones of histological preparations, by means of the X-ray electron probe.

Procedure. 10–15 μm thick histological sections are prepared conventionally and mounted on glass slides with gelatin. The sections are deparafinized and covered with a thin Vestopal W film. After polyester hardening for 12 h at 60 °C, the sections are covered with carbon film by means of a Edwards vacuum coating unit model E 12 E.

Operations of the electron probe. An AMX electron probe (ARL – Glendale, Calif.) is used at an accelerating potential of 20 kV with a specimen current on brass, of 25 nA. A LiF crystal spectrometer is focused on Osmium Lα, Iodine Lα and Zinc Kα rays by means of an aqueous solution of OsO_4 (1%) and ZnI_2 (4%) evaporated on a slide and covered by Vestopal W. Discrimination of Zn Kα and Os Lα spectra is distinct with this technique (Figure 1).

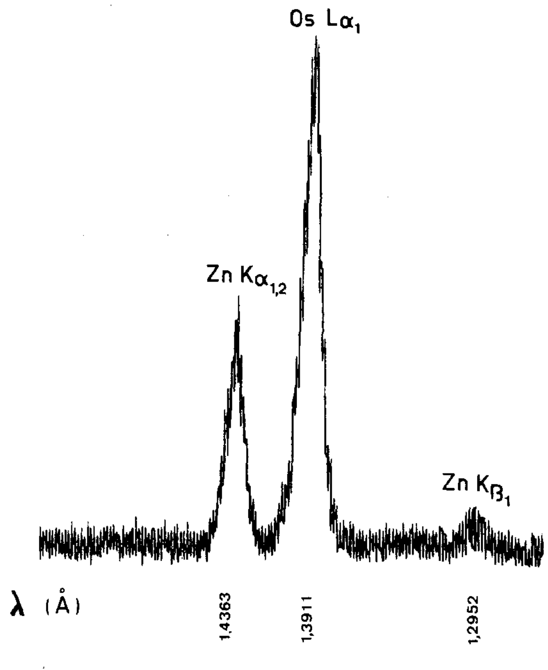

Fig. 1. Discrimination of Zn and Os in X-ray spectrometric analysis. Angström wavelenghts shown for each peak.

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[1] M. MAILLET, C.r. Soc. biol., Paris *153*, 939 (1959).

Results. We have observed several 200 × 200 µm fields of the muscular byssus complex innervation of *Mytilus edulis* L. (Mollusca, Lamellibranchiata) with significant results. One characteristic field is presented here (Figure 2, M). This same zone has been scanned for its Osmium (Figure 2, Os) and Zinc (Figure 2, Zn) distribution. All 3 images clearly represent superimposable patterns. The CHAMPY-MAILLET stain contains evidently Zn next to Os. On the other hand, it has not been possible to obtain any significant X-ray image of Iodine.

Moreover, X-ray intensity profiles were run across the zones to show the relative distribution of Os, Zn and I (Figure 2, P). To generate these profiles, the focused beam of the probe has been traversed across the 200 µm field at a constant rate along the lines AB and CD. During these traverses, the X-ray intensity of each element is recorded; the ordinate of the profiles is proportional to the relative amount of the chosen elements. The distribution of Os and Zn in both profiles is remarkably similar; obviously the same histological structures take compound Zn-Os stain.

On the other hand, in spite of a more sensitive recording threshold of the probe, no significant profiles are recorded for Iodine; the registration does not exceed the level of the background.

Discussion. MAILLET[2] explains the staining by means of an osmical iodate complex fixation at sites of several cellular unsaturated proteolipids, biogenic amines and their chemical precursors in somatic or peripheral extensions including terminal nervous structures; he considers Iodine to be essential for the development

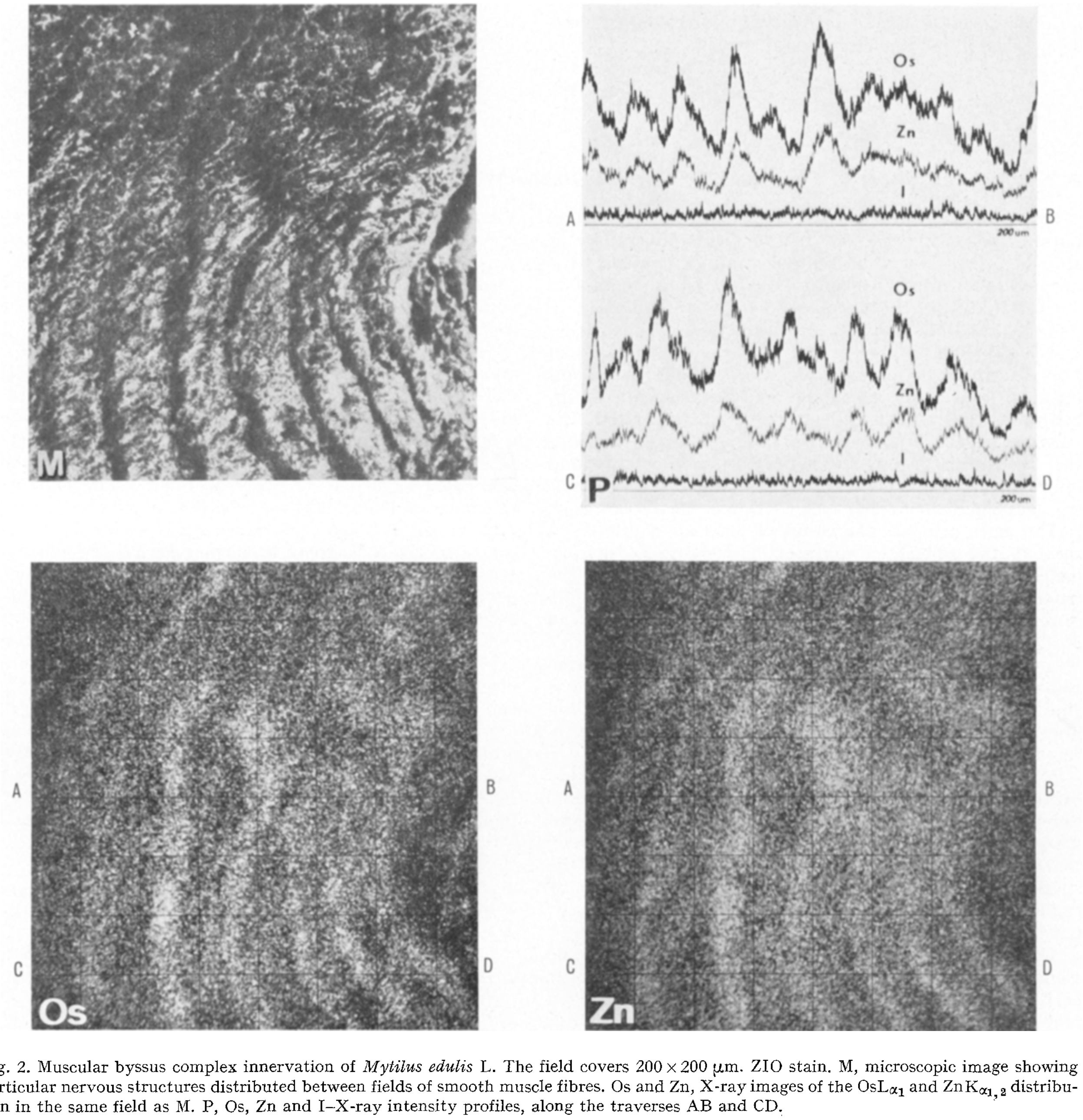

Fig. 2. Muscular byssus complex innervation of *Mytilus edulis* L. The field covers 200 × 200 µm. ZIO stain. M, microscopic image showing particular nervous structures distributed between fields of smooth muscle fibres. Os and Zn, X-ray images of the $OsL_{\alpha 1}$ and $ZnK_{\alpha 1,2}$ distribution in the same field as M. P, Os, Zn and I–X-ray intensity profiles, along the traverses AB and CD.

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[2] M. MAILLET, C.r. Ass. Anat., Paris *140*, 233 (1968).

of the stain. He adds that best results appear when divalent iodate cations such as Zn, Cd or Ca were simultaneously present with OsO_4. Nothing is found in the scientific literature to show whether these divalent cations are part of the dense black organometallic complex.

Our interpretation of the reducing process calls for complex cations, Os-Zn^{x+}, to oxidize double bonds from organic reducing substrates in the cells. It may be possible that Iodine is required for the development of the staining reduction, but this element is apparently removed from the preparations by subsequent washings of the histological procedure.

Conclusion. Structures stained by ZIO technique contain both Zn and Os. The distribution of both elements is similar and the ratio Zn/Os is constant.

By substitution of divalent cation such as Zn in the place of K in CHAMPY's, OsO_4-KI, staining fixative mixture, MAILLET affords a more constant staining technique where Os and Zn are associated and participate in the formation of some organometallic complex at the level of several histological and cytological structures, such as nervous peripheral structures[3,4].

Résumé. La sonde électronique de Castaing nous a permis de déceler la présence de zinc simultanément à celle de l'osmium au niveau des sites de réduction du fixateur-colorant histologique de CHAMPY-MAILLET. L'iode ne fait pas partie du complexe organométallique colorant.

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A Note on the Effect of Chemical Treatments in the Mineralogical Studies of Sediments

Several chemical treatments are commonly applied to natural sediment samples before their mineralogy is studied using X-ray and other analytical tools. The method often employed consists of treating the sediments with Na-citrate, Na-bicarbonate and Na-dithionite to remove metallic coatings, by reducing Fe and other metals to their soluble states, and then with H_2O_2 of breaking up the organics by oxidation[1]. Similar treatment has also been employed in the study of suspended sediments[2,3]. It is assumed during such treatment that the silicate minerals in the sediments are unaffected and that the separation of the sediments into non-detrital and detrital fractions is effective[1]. However, instances of the silicates being affected by such treatment are also known[4].

This note presents the result of Mössbauer effects on treated and untreated samples. The sediment sample, collected from the Atlantic Ocean off the mouth of the Amazon River, consists of equal amounts of kaolinite, illite and montmorillonite[2]. The source for γ-radiation in Mössbauer effect was Co^{57} in stainless steel. Metallic Fe was used as reference standard. The data obtained from the multichannel analyzer was computer fitted to obtain the velocity-absorption (intensity) spectra. The position, amplitude and width were allowed to vary independently in order to obtain the best fit.

Mössbauer effect was observed on both 'treated' and 'untreated' sediment. The sediment used has equal amounts of the 3 major clay minerals in ratios approximately the composition of world average suspended matter carried by rivers into the oceans[5]. Hence the single sediment employed is representative of all clay mineral suites delivered to world oceans at the present time.

The spectra from the Mössbauer effect are shown in the Figure and the data are presented in the Table. The upper and lower spectra in the Figure correspond to untreated and treated sediments respectively. From the computer calculated data shown in the Table, it is clear that chemical treatment affects the basic structure of the silicates, in addition to removing some of the Fe in coatings as reflected in changes in the intensity for Fe^{+3}-peaks. Peak A in the spectra represents the sum of 2 overlapping peaks identical with B and C (intensity of A = intensity of B + intensity of C). The spectra correspond to the presence of Fe^{+3} in hydroxide coatings, octahedral and tetrahedral lattice sites in clays and Fe^{+2} in octahedral sites in clays[2,6].

Treatments for removal of coatings are supposed to eliminate most of the Fe^{+3} from the sediments, since Fe in coatings far exceed Fe^{+3} in clay lattices, but there is only a very small change in the intensity of Fe^{+3} peaks after treatment; hence the treatment method also appears to be inefficient. Changes in the intensity of Fe^{+3} peaks have also been reported by other workers[6]. The enhanced intensity of Fe^{+2} peaks may be due to increase in Fe^{+2} relative to Fe^{+3} by removal of Fe^{+3}, as shown by the intensity ratios. Changes in various parameters observed for Fe^{+3} peak suggest the possible attack of chemicals on the octahedral Fe^{+3} in clays and also on octahedral Fe^{+2} [4].

Some of the Fe^{+2} in the octahedral sites could have been displaced by small changes in the lattice parameters after the chemical attack; Fe^{+3} in the octahedral sites could have been reduced during the treatment to Fe^{+2}, and become soluble, and thus removed from the silicates along with the Fe^{+3} in the coatings. In the sediment examined, kaolinite and illite fraction have probably no Fe^{+2} or Fe^{+3} in the lattices at all, but montmorillonite is likely to contain both Fe^{+3} and Fe^{+2} in the lattices as

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[1] M. L. JACKSON, *Soil Chemical Analysis* (University of Wisconsin, Madison 1969).

[2] R. J. GIBBS, Bull. Geol. Soc. Am. *78*, 1203 (1967).

[3] J. W. PIERCE, D. D. NELSON and D. J. COLQUHOUN, *Shelf Sediment Transport* (Ed. D. J. P. SWIFT; Downden and Co., New York 1972), p. 281.

[4] N. H. GRANGAS, A. SIMOPOULOS, A. KOSHIKAS, N. J. YASSAGLOU and S. FILLIPAKIS, Clay Miner. Bull. *21*, 151 (1973).

[5] R. M. GARRELS and F. T. MACKENZIE, *Evolution of Sedimentary Rocks* (Norton and Co., New York 1971).

[6] E. C. WEAVER, J. M. WAMPLER and T. E. PECUIL, Science *156*, 504 (1967).