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The Curarelike Effect of Thiamine Hydrochloride Solution on Vagal Inhibition of the Frog Heart

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The curarelike effect of thiamine hydrochloride upon the frog has been described by Smith *et al.* (*2*). In their study, 2000 mg of thiamine hydrochloride per kg of body weight was injected into the ventral lymph sac of adult frogs. By exposing both gastrocnemius-sciatic systems and ligating one of these muscles from the systemic circulation prior to the injection, it was possible to demonstrate that stimulation of the sciatic nerves produced contraction of the gastrocnemius muscle only on the ligated side. The same phenomenon was observed with injections of 80 units intocostrin per kg body weight.

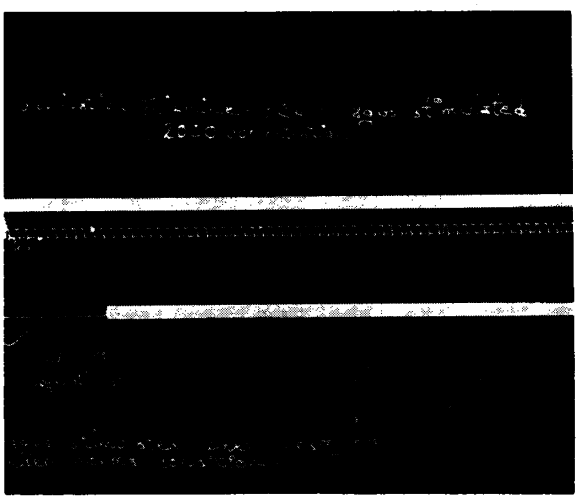

FIG. 1.

Inhibition of the vagus effect on the frog heart by addition of thiamine chloride to a perfusion fluid has been shown by Gross (*1*).

To determine if thiamine hydrochloride would also produce a curarelike effect on vagal inhibition of the frog heart, solutions buffered at pH 7.2 and ranging in concentration from 1: 5000 to 1: 1000 were applied to the exposed heart. The right cardiac vagus was then stimulated with induction shocks. At dilutions of thiamine hydrochloride greater than 1: 2000, vagal stimulation was effective in stopping the heartbeat. Dilutions of 1: 2000, however, resulted in complete blocking of the vagus inhibition (Fig. 1). Application of approximately 1 ml (500 μg) was effective in accomplishing this result. The vagus effect was again restored after flushing with Ringer's solution and allowing a 3-min interval.

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On the Existence of Bonds between Ossein and Inorganic Bone Fraction

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The problem of the existence of bonds between the ossein and the inorganic fraction of bone is as yet unsettled: Is the ossein physically mixed or chemically bound with the inorganic bone fraction?

Caglioti (*4*) has shown that the bone x-ray pattern gives evidence of the existence of a semicombined lattice formed by ossein and the inorganic fraction.

On the other hand, Dallemagne and Mélon (*5*), from quantitative research upon the optical properties of ox bone, claim to have demonstrated that its composition obeys Wiener's law (*10*) concerning form birefringence of rodlet composite bodies. Consequently, the organic and inorganic fractions of the ox bone appear to be physically mixed.

In previous research on the optical properties of human bone (*1, 2*), I pointed out that the conclusions attained by the latter authors do not seem wholly acceptable for the following reasons: (*a*) Dallemagne and Mélon do not take any account of the birefringence of ossein itself; and (*b*) technical difficulties do not allow precise measurements of the refractive index of ossein by means of the Becke and Schroeder van der Kolke methods, so that the value found for ossein ($n = 1.385$) actually appears to be excessively low.

The present note is an attempt to bring further knowledge to bear on this subject. Details of this investigation will be published elsewhere.

The ossein (ground substance + fibers), like the inorganic fraction, follows Wiener's law of form birefringence. This property seems to be related to the submicroscopic spaces occupied by the inorganic fraction. Contrary to Baud and Dallemagne's statements (*3*), it is possible to obtain the ossein form birefringence curve without any evident anomalies in its general behavior. This is easily obtainable (provided that the imbibition of

TABLE 1

Refractive index of imbedding fluids	Form birefringence (femoral diaphysis)		
	Ox	Man (Case C.A., ♂, 25 years)	
	Ossein	Ossein	Inorganic fraction
1.333	0.0048	0.0043	0.0095
1.365			0.0069
1.385	0.0028	0.0026	
1.395			0.0049
1.405			0.0039
1.422			0.0024
1.426		0.0014	
1.428	0.0015		
1.443	0.0011	0.0013	
1.447			0.0019
1.465			0.0007
1.466	0.0007	0.0006	
1.487			−0.0008
1.492	0.0005	0.0004	
1.508	0.0005	0.0004	
1.541			−0.003
1.562			−0.0036
1.596			−0.0037
1.659			−0.0028
1.743			0.0014

the material is kept between the physiological limits) by washing and neutralizing the decalcified bone slices in normal saline and by choosing fluids (in which the slices have to be imbedded) of different refractive index so that these fluids have the same imbibition power for ossein as does water or normal saline. In such a way,

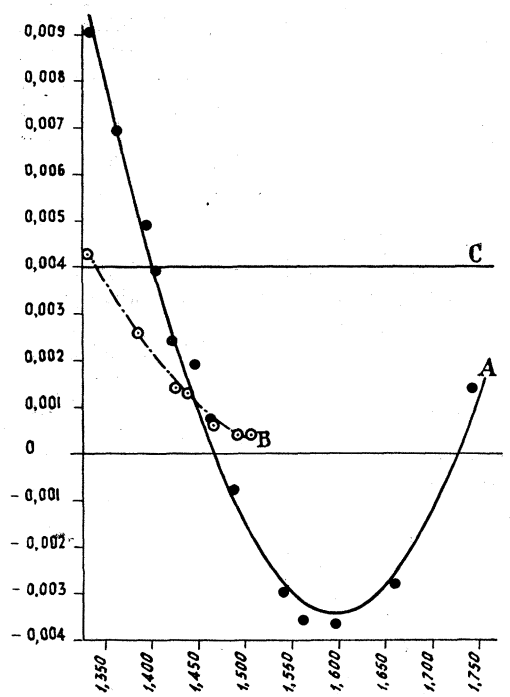

FIG. 1. Femoral diaphysis of case C.A., ♂, 25 years. *A*, Form birefringence curve of the inorganic bone fraction; *B*, Form birefringence curve of the ossein; *C*, Birefringence of total bone.

anomalies of the curve, due to swelling or shrinking of the material, are avoided. In order to satisfy this last condition, use was made of different dilutions of glycerol ($n = 1.470$) in distilled water or normal saline (Schmidt [*9*], Frey-Wyssling [*7*]). Solutions of cadmium chloride in glycerol up to saturation were used in order to have fluids of higher refractive index (i.e., up to 1.508), according to Schmidt (*9*).

The retardations were measured by means of a Berek compensator, and the double refraction power was calculated by means of the analogous equation suggested by Dallemagne and Mélon (*5*) for inorganic bone fraction:

$$B_d = \frac{B_t \cdot R_d}{R_t}$$

where B_d represents the birefringence of decalcified bone, B_t the double refraction of total bone: 0.004 for ox bone (Mélon and Dallemagne [*8*]) and human bone (Ascenzi [*1, 2*]), R_t the retardation measured in total bone slices, and R_d the retardation in decalcified slices.

The average birefringence for each imbibition fluid, as calculated from a sufficiently large number of measurements (50), allows one to draw a perfectly shaped form birefringence parabolic curve.[1] The values for human and ox bone thus found are reported in Table 1, together with the values of form birefringence of the human inorganic fraction (Ascenzi [*1*]). In Fig. 1 the curves of the form birefringence of the human inorganic bone fraction and of ossein are shown. The unavoidably circumscribed interval of the refractive index of the fluids employed prevents the study of the entire ossein curve.

As the analysis shows, this curve does not represent the form birefringence of the physiologically swollen ossein when different fluids have penetrated into the submicroscopic spaces formerly occupied by inorganic fraction. Rather, the curve represents the form birefringence of the protein frame in relation to the fluids that have penetrated into the spaces created by the removal of the inorganic fraction and also into the spaces previously occupied by the water of imbibition.

As a matter of fact, when we apply the equations obtained by interpolating the values listed in Table 1,

$$y = 0.224206 - 0.290512\,x + 0.094195\,x^2 \quad \text{(human ossein)}$$
$$y^1 = 0.298175 - 0.392353\,x_1 + 0.129255\,x_1^2 \quad \text{(ox ossein)}$$

the coordinates of the minimum turn out to be

$$x = 1.542 \qquad y = 0.0002$$
$$x_1 = 1.517 \qquad y_1 = 0.0004.$$

It is clear that the abscissa corresponds, not to the mean refractive index of the physiologically swollen ossein, but rather to that of dry ossein (1.547, according to Dallemagne and Mélon). Hence, it would seem more reasonable to consider the refractive index as the index of the protein ossein frame. Similarly, the ordinate of the minimum point of the curve measures the intrinsic birefringence of the protein frame.

Therefore, we are justified in holding that the ossein at its physiologically swollen state in the bone consti-

[1] Or hyperbolic curve, according to Frey-Wyssling (*6*).

tutes a composite body (protein frame + water of imbibition) and has the birefringence of rodlets, although experimentally not measurable. These considerations suggest that bone is really a complex system. Hence, it would be dangerous to apply to bone (as Dallemagne and Mélon do) a reasoning that is valid only for a two-component composite body.

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Brucella Ring Test Antigen Prepared by Reduction of a Tetrazolium Salt

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Brucella Ring Test is the name given the phenomenon which occurs when stained *Brucella* antigen is added to whole milk. If the milk contains *Brucella* agglutinins the stained antigen is agglutinated and rises with the cream to give an intensely colored cream layer, or ring. This test was first described by Fleischhauer (*2*) and is rapidly coming into general use as a quick and simple method of testing herds of cattle for evidence of brucellosis.

Present methods of staining antigen make use of hematoxylin, which frequently results in antigen unsatisfactory with respect to sensitivity, specificity, color intensity, and keeping quality. In addition the procedures are laborious and critical.

Tetrazolium salts have been used for staining plant and animal tissues. Several of these compounds were used to stain *Brucella* antigen, and the one found most suitable is 4,4′-bis(3,5-diphenyl-2-tetrazolinium)-biphenyl dichloride. This compound is reduced by living cells to an intensely colored violet-blue formazan. It appears that this reduction takes place inside the cell, and hence the antigenic specificity of the cell surface is not altered.

To prepare *Brucella* ring test antigen the organisms are grown using the method of Brown and Wood (*1*). To the heavy suspension of living cells in broth obtained by this method an aqueous solution of 4,4′-bis(3,5-diphenyl-2-tetrazolinium)-biphenyl dichloride is added to give a final concentration of one part in 16,000. This mixture is incubated at 37° C for 4 hr to permit the organisms to reduce the compound and thereby become stained. The organisms are then killed by heating to 60° C for 1 hr. The antigen is then concentrated by centrifugation and resuspended to the desired density in 0.85% NaCl solution containing 0.5% phenol as a preservative.

More than 100 different lots of antigen have been prepared over the last 2 years using this method, and all have been of uniform color intensity, specificity, and sensitivity. This antigen has remained stable over prolonged periods and under normal conditions of use and storage.

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Protection of Mice against X-Radiation by Thiourea

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It was reported previously (*4*) that thiourea protected desoxyribosenucleic acid (DNA) against x-ray depolymerization in aqueous solution and *in vivo*. Barron *et al.* (*1*) have reported that sulfhydryl-containing enzymes in aqueous solution were inactivated by x-radiation because of the oxidation of the sulfhydryl group and that the enzymes were reactivated by the addition of glutathione. Ephrati (*2*) found that the inactivation of tetanus toxin and of staphylococcus hemolysin by x-radiation in aqueous solution was inhibited by the presence of reducing agents such as ascorbic acid and glutathione, whereas oxidizing agents did not inhibit the action. Forssberg (*3*), however, found that reducing agents (cystein) enhanced the inactivation of catalase by x-radiation, whereas oxidizing agents (cystine) inhibited this action. Patt and his coworkers (*5*) have recently found that cysteine decreased the mortality of x-rayed rats, whereas cystine was ineffective. The protection by thiourea of a vital cellular constituent, such as DNA, therefore might be assumed to affect the mortality of x-rayed animals.

Male, white mice (Detwiler) weighing 18–22 g were allowed Fox Food Blox (Allied Mills) and water ad libitum. The mice were irradiated in groups of 20–25 with 650 r given at the rate of 100 r/min. The constants of the x-ray machine were 250 kv and 15 ma. A copper filter ½ mm thick was used, in addition to the inherent filtration of 3 mm of aluminum.

In a preliminary experiment an aqueous solution of thiourea (90 mg/ml) was injected intraperitoneally in a single dose (1,280 mg/kg) 5 min before irradiation. In a subsequent experiment thiourea was administered in the drinking water in a concentration of 1% for a period