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## MAIN SUBJECT HEADING:

AN  
ANALYTICS**HU**  
HUMAN  
EFFECTSAT  
ANIMAL  
TOXICITYIH  
WORKPLACE PRACTICES-  
ENGINEERING CONTROLSM  
MISCELLANEOUS

SECONDARY SUBJECT HEADINGS: AN HU AT IH M

Physical/Chemical Properties

Review

Animal Toxicology

Non-occupational Human  
Exposure

Occupational Exposure

Epidemiology

Standards

Manufacturing

Uses

Reactions

Sampling/Analytical Methods

Reported Ambient Levels

Measured Methods

Work Practices

Engineering Controls

Biological Monitoring

Methods of Analysis

Treatment

Transportation/Handling/  
Storage/Labeling

isocalloxazine part of the molecule. It has a partition coefficient of 0.54 between benzyl alcohol and water compared with 3.2 for riboflavin, 0.010 for flavin mononucleotide and 0.004 for flavin adenine dinucleotide<sup>1</sup>, of which fact use is made during the isolation of the material. Comparison of the absorption spectra of riboflavin and of the flavin under investigation reveals no significant differences.

Recently a new flavin, flavin-X, has been reported in the literature<sup>2</sup>, but the possibility that the flavin here described may be identical with flavin-X is eliminated by the fact that it does not contain phosphorus, whereas flavin-X is reported to be a flavin dinucleotide.

The possibility that this new flavin may be lyxoflavin<sup>3</sup> has not yet been excluded; it is, however, considered unlikely that so small a change in structure can account for so many differences in properties. Investigations are continuing on the nature of the compound, and they will be published in full elsewhere.

I am indebted to Dr. M. Dixon for his advice and encouragement, and to the Medical Research Council for a grant.

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May 13.

<sup>1</sup> Crammer, J. L., *Nature*, **161**, 349 (1948).

<sup>2</sup> Watanabe, O., and Christian, W., *Biochem. Z.*, **229**, 156 (1928).

<sup>3</sup> Linnarsson, A., *Rec. Trav. chim.*, **58**, 290 (1939). Jessup, O. A., Lowry, O. H., and Love, R. H., *J. Biol. Chem.*, **180**, 735 (1949).

<sup>4</sup> Sanadi, D. R., and Huebner, F. M., 117th Meeting of the American Chemical Society, Abstracts of Papers, p. 60c (1950).

<sup>5</sup> Pallares, E. S., and Garza, H. M., *Arch. Biochem.*, **22**, 63 (1949).

### Anticoagulant Activity of the Indian Cattle Leech

The anticoagulant activity of leeches has been known for a long time. Haycraft<sup>1</sup> obtained a saline extract of the anticoagulin from the head of a leech, and found that a dose of 0.01 mgm. would prevent the normal coagulation of 50 ml. blood for four and a half hours. Various investigators have since employed different techniques in their study of the anticoagulant action of leech extract. In our experiments with the common Indian cattle leech (*Hirudinaria*), an extract of the anticoagulant substance was prepared from the first eight segments of the body of the leech. The tissues were pounded with pure quartz sand in a mortar and the solution filtered through a cotton plug, freed from protein and buffered to pH 7.2 with Sorensen phosphate buffer.

Preliminary observations on the effect of freshly prepared extract were made on human and other mammalian blood *in vitro* and on white mice (weighing 60-100 gm.) *in vivo*. The coagulation time recorded in each experiment was much prolonged, and no case of haemolysis of blood, as noticed by some previous investigators, was met. The extracted solution in most cases seemed to retain its anticoagulant property up to three weeks when stored at room temperature, with a maximum range between 95° and 100° F.

Furthermore, it was found that the fresh extract was absolutely non-toxic (unlike the one found by Marshall<sup>2</sup>) when administered to white mice by the intravenous, intramuscular or oral route.

There was a considerable increase in blood coagulation-time in white mice when freshly prepared extracts were administered by an intravenous or an oral route. The results of intramuscular injection in

white mice were variable and require further elucidation.

The two important anticoagulants which have been used therapeutically are heparin and dicumarol. Whereas heparin is only effective when given intravenously, dicumarol is useful only when given orally. The extract from the Indian leech appears to act as an effective anticoagulant both by the intravenous and oral routes.

Further work is in progress to evaluate the comparative yield and the therapeutic anticoagulant effect of the extract from *Hirudinaria*.

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<sup>1</sup> Haycraft, J. B., *Arch. Exper. Pathol. u. Pharm.*, **18** (1884).

<sup>2</sup> Marshall, J., *J. Pharm.*, **7** (1915).

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### Dielectric Properties of the Human Body for Wave-lengths in the 1-10 cm. Range

A SUMMARY of the results of measurements of the dielectric properties of a representative selection of body tissues at a wave-length of 3.18 cm. has already been published<sup>1</sup>. These measurements have now been extended to two other wave-lengths, namely, 1.27 cm. and 10.0 cm., the method used being similar to that already described. All specimens, with the exception of bone, were taken from surgical operations, and measurements were made in each case with the minimum of delay and at a controlled temperature of  $37.0 \pm 0.5^\circ \text{C}$ .

In Table 1, the results for these wave-lengths are tabulated, each being expressed in terms of an absorption constant ( $\alpha$ ) and a phase constant ( $\beta$ ) for the tissue filling the aperture of a wave-guide appropriate to the wave-length used. The figures given are the average values obtained from two or more measurements on sections from one source, and the averages for sections from different sources are included, where possible, to indicate the variations encountered in practice. The measurement accuracies are estimated as  $\pm 10$  per cent in  $\alpha$  and  $\pm 5$  per cent in  $\beta$  for 1.27-cm. wave-length and  $\pm 5$  per cent in  $\alpha$  and  $\pm 2$  per cent in  $\beta$  for 10.0-cm. wave-length.

In Table 2, the real and imaginary components of the complex dielectric constant  $\bar{\epsilon} = \epsilon' - j\epsilon''$ , calculated from the measured constants for a number of tissues, are compared at the three wave-lengths 10, 3.18 and 1.27 cm.

As with the 3.18 cm. wave-length, the measured constants for the two wave-lengths 1.27 and 10.0 cm. may be compared with those for water, the latter being present in a high percentage in all the tissues other than fat and bone. Determined in the same equipment, the constants for water at  $37^\circ \text{C}$ . were  $\alpha = 12.3$ ,  $\beta = 34.2$  for 1.27-cm. wave-length, and  $\alpha = 0.3$ ,  $\beta = 5.4$  for 10-cm. wave-length.

The general conclusions to be drawn from the measurements at the two wave-lengths are the same as those for 3.18-cm. wave-length, namely, that many of the body tissues behave very similarly to water in respect of their dielectric properties, that fat and bone, having much lower water contents, are relatively transparent to the radiation and that malignant and normal tissues do not differ significantly. One difference between tissue behaviour relative to water

Table 1

Specimen	$\lambda = 1.27 \text{ cm.}$		$\lambda = 10.0 \text{ cm.}$		Remarks
	$\mu$ (radius/cm.)	$\beta$ (radius/cm.)	$\alpha$ (radius/cm.)	$\beta$ (radius/cm.)	
Whole blood	0.5	0.1	0.65	4.0	Unconjugated by 3/200 mgm. heparin
Blood serum	0.7	0.1	0.81	5.0	Centrifuged specimen
Skin	0.4	0.2	0.81	5.1	Breast specimens free of areolar tissue
	0.3	0.2	0.65	4.6	
Fat	1.0	0.2	0.73	4.6	Breast specimens free of areolar tissue
	1.0	0.2	0.20	1.70	
Bone	1.0	0.2	—	—	Post-mortem specimens. Sections taken at different depths from the surface
	1.0	0.2	—	—	
Cross-section of Breast	1.0	0.2	0.73	4.8	All specimens confirmed scirrus carcinoma by histological examination
	1.0	0.2	0.60	5.0	

Table 2

Specimen	$\epsilon'$ (relative to air)			$\epsilon''$ (relative to air)		
	10.0 cm.	1.27 cm.	1.27 cm.	10.0 cm.	1.27 cm.	1.27 cm.
Whole blood	15	25	22	15	23	20
Blood serum	14	25	25	12	21	20
Skin	20	25	24	16	18	13
Fat	10	10	10	1.0	0.65	1.1
Bone	10	10	10	—	1.45	1.1

... wave-length and at the shorter wave-lengths is that the absorption constant  $\alpha$  tends to have a value approximately double that of water, indicating the significant contribution of the ionic conductivity absorption. Assuming equal contributions to total absorption at this wave-length by the water molecules and by ionic conductivity, a value of the order of  $10^4$  ohms per cm.<sup>2</sup> is obtained for the conductivity, which is in agreement with the values obtained from measurements at longer wave-lengths (summarized by Osler and T. S. Encland). The ionic conductivity contributions to absorption in such tissues at wave-lengths of 10.0 cm. and 1.27 cm. would appear, by similar calculations, to be about 10 per cent and 2 per cent respectively, and would not be immediately evident as measured constants.

The assistance of Dr. N. A. Sharples in selection and preparation of specimens is gratefully acknowledged.

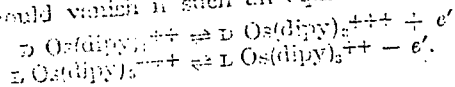
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Received T. S. and Sharples, N. A., *Nature*, 163, 487 (1949).  
Kosman and Michalek, "Technic of Electrotherapy", 456 (1945).

### A Reaction for the Study of the Kinetics of Electron Transfer

The isolation of stable optical forms of the tris (2,2'-dipyridyl) osmium II ion,  $Os(dipy)_3^{2+}$ , and of the tris (2,2'-dipyridyl) osmium III ion,  $Os(dipy)_3^{3+}$ , is a unique demonstration of the dynamic equilibrium between the oxidized and reduced forms of an ion. It is evident that when the more stable form of the osmium II complex ion is mixed with an equivalent amount of the *leuco* form of the osmium III complex ion, the optical activity of both should vanish if such an equilibrium exists.



The experiment was carried out by mixing 10 mgm. of D, tris (2,2'-dipyridyl) osmium II perchlorate with 10.0 mgm. of L, tris (2,2'-dipyridyl) osmium III perchlorate in 20 ml. of water at 20° C. After two minutes, the osmium II complex was precipitated by the addition of 20 per cent sodium perchlorate solution, leaving a pink solution of the osmium III complex. Both the precipitate and the filtrate were found to be inactive.

Essentially the same experiment has been carried out in the studies of the ferrous/ferric and the tris ethylene diamine cobalt II/tris ethylene diamine cobalt III equilibria with radioactive iron<sup>57</sup> and cobalt<sup>60</sup>. However, the present experiment may also be used to determine the rate of electron transfer under varying conditions of temperature, concentration, and dielectric constant of the solvent without the necessity of altering the conditions by separation of the oxidant and the reductant.

Tris (2,2'-dipyridyl) osmium II perchlorate has  $[\alpha]_{546}^{25} = 2200^\circ$ , while for tris (2,2'-dipyridyl) osmium III perchlorate,  $[\alpha]_{546}^{25} = 206^\circ$ . The mixture of equivalent amounts of the D osmium II complex and L osmium III complex, at the instant of mixing, is therefore dextrorotatory, and the loss of activity consequent on electron transfer can be followed in a polarimeter. The intense green colour of the reduced form of the complex necessitated the use of very dilute solutions, and the maximum angle of rotation that could be comfortably observed was about 0.08°.

With the optimized solution  $5 \times 10^{-4} M$  with respect to both ions, the exchange was complete in less than 15 sec. at 25° C., but required approximately 65 sec. at 10° C. and 95 sec. at 5° C. The rate at 10° C. was very much greater in 2 N ammonium nitrate solution, but much slower in acetone, than in water. Both these effects are obviously related to the dielectric constant of the solution.

It is hoped to study quantitatively the effects of various factors, particularly concentration, with a photo-electric polarimeter.

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<sup>1</sup> Bursell, F. H., Dwyer, F. P., and Gyafas, E. C., *J. Chem. Soc.*, 533 (1950).  
<sup>2</sup> Dwyer, F. P., and Gyafas, E. C. (unpublished work).  
<sup>3</sup> Van Alben, J., *Amer. Chem. Soc.*, 70, 583 (1948).  
<sup>4</sup> Lewis, N. D., and Correll, C. D., Brookhaven Conf. Report. Chemistry, No. 2, 131 (1948).