2. Nach Beschallung mit 2000 Hz und 140 db während 4–6 h

a) *Verhalten von Kalium.* Am auffallendsten ist der ungefähr dreifache Anstieg des Kaliumgehalts in der zweiten Windung der vestibulären Perilymphe, das heisst in Höhe der Tonperzeption, während die Kaliumwerte weder in der ersten, dritten und vierten vestibulären Windung noch in einer der tympanalen Windungen nennenswerte Änderungen erfahren.

Dieser auffallende Unterschied kommt mit K-42 nicht zur Darstellung.

Der totale Kaliumgehalt der Endolymphe wie der K-42-Austauschwert nehmen in der zweiten Windung leicht ab (15–20%), während in den übrigen Windungen keine Veränderungen zu erkennen sind.

b) *Verhalten von Natrium.* Der Natriumgehalt nimmt in beiden Perilymphräumen nach Beschallung leicht zu, apikal stärker als basal. Wesentlich ausgesprochener sieht man diese Zunahme in den Na-24-Austauschwerten, und zwar sowohl in der Peri- wie in der Endolymphe (durchschnittlich um 15% in der 1. Windung, um 20–40% in der 2. Windung, 40–60% in der 3. und über 60% in der 4. Windung).

Die übrigen Partien der Cochlea, die Basalmembran und die knöcherne Kappe zeigen gleichgerichtete Veränderungen mit Ausnahme der Stria vascularis, deren Na-24-Austauschwerte selbst abnehmen.

Folgerungen. Trotz der Diffusionsmöglichkeiten ergeben sich erstaunlich grosse Unterschiede einerseits zwischen der tympanalen und vestibulären Perilymphe, andererseits zwischen den verschiedenen Windungen. Dieser Befund, erhoben im Ruhezustand, wird nach Einton-Reizung noch augenfälliger.

Da nach histologischen Befunden Töne von 2000 Hz in der zweiten Windung perzeptiert werden, finden die chemischen Veränderungen in der zweiten Windung nach Ertaubung eine Erklärung.

Ausserdem scheinen sowohl die höheren Kaliumwerte in der vestibulären Perilymphe, die in der Basalwindung besonders hervortreten, wie auch ihr deutlich erhöhter Kaliumgehalt in der zweiten Windung nach Beschallung ein Hinweis zu sein, dass die Reissnersche Membran zu gegebener Zeit zum mindesten partiell semipermeabel ist.

Weit interessanter wird es sein, den Effekt zu verfolgen, den ein Einzelton von verschiedener Intensität an chemischen Veränderungen in der Peri- und in der Endolymphe auslöst.

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Summary

A freezing method is described which permits the measurement of electrolytes and enzymes in the peri- and endolymph from a single turn of the cochlea of guinea pig at a known time. The perilymphs of the scala vestibuli and the scala tympani are different, especially in the first basic turn. This difference may be produced by a partial semipermeability of the Reissner's membrane. After producing deafness by a sound of frequency 2000 c/s, the potassium (the total potassium level detected chemically and by the use of potassium-42) increases in the perilymph of the second turn only, and decreases slightly in the endolymph of the same turn. In the other turns, the potassium does not change.

This method permits the analysis of chemical changes due to the stimulus of single sounds.

The Effect of Methyl Alcohol on the Conversion of Thiamine to Thiochrome[1]

Oxidation products of thiamine have been studied extensively by BARGER *et al*[2], JANSEN,[3] ZIMA and WILLIAMS[4], SYKES and TODD[5], and MIZUHARA and ARATA[6]. The major product of alkaline oxidation of thiamine by $K_3Fe(CN)_6$ is thiochrome. JANSEN[3] using the thiamine-thiochrome conversion as a quantitative determination of thiamine, suggested the addition of CH_3OH to the oxidation mixture to prevent «harmful» action of the excess ferricyanide. Later investigators apparently omitted CH_3OH but obtained only a 65–67% conversion[7–9]. Since no reliable data (except JANSEN's work in 1936) on the influence of CH_3OH on the thiamine-thiochrome conversion seemed to be available, studies were made to determine the effect. The data indicate that addition of 25% (vol/vol) CH_3OH to the alkaline oxidation mixture results in better than 90% conversion in the concentration range usually employed.

Procedures. Standard stock solutions contained 100 mg% thiamine hydrochloride (Merck and Company) or 100 mg% thiochrome (Nutritional Biochemicals) in 0·1N HCl. These were diluted with 0·1N HCl to give 0·1, 0·2, 0·4, 1·0, 200 and 1000 µg/ml of solution.

To 1 ml samples of the diluted 0·1N HCl thiamine solutions were added 0 to 3 ml of redistilled CH_3OH, 3 ml of 0·3% $K_3Fe(CN)_6$ in 15% NaOH and distilled water to make a total of 8 ml of solution. The same reagents were added to the thiochrome solutions. 15 ml of redistilled isobutanol were then added and the mixtures shaken vigorously for $1^1/_2$ min. Blanks were run by omitting $K_3Fe(CN)_6$ from the reaction mixture. After centrifuging at 500–600 r.p.m. for 45 sec, 10 ml of the top isobutanol layer (or an appropriate dilution of this layer with isobutanol) was pipetted into a 19 × 105 mm cuvette and the fluorescence measured in a Coleman photofluorometer using 0·01 mg% quinine sulfate in 0·1N H_2SO_4 as a reference standard. Thus the fluorescence of thiochrome derived from a known amount of thiamine in the reaction mixture was compared to the fluorescence of a comparable amount of thiochrome treated with the same reaction mixture. To investigate the validity of this procedure, fluorometric readings of thiochrome solutions were compared as follows:

(a) Thiochrome dissolved in the reaction mixture as described above, containing 0 and 2 ml respectively of CH_3OH and extracted into isobutanol by shaking for $1^1/_2$ min.

(b) Thiochrome dissolved directly in isobutanol.

The results of this series are given in Table I. The data indicate a somewhat lower recovery of thiochrome when CH_3OH is omitted from the reaction mixture. However,

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Tab. I. Fluorometer Readings for 0.3 μg Thiochrome Using Quinine Standard Setting of 50 (see text). Average Readings of 6 Series of Experiments. SDM Values Given.

	Thiochrome in 8 ml reaction mixture + 15 ml isobutanol		Thiochrome in 15 ml isobutanol
Ml CH_3OH in reaction mixture	2	0	—
Final volume of isobutanol phase (ml)	17.6	16.0	15.0
Average reading corrected to volume of 15 ml isobutanol phase	63.4 ± 0.8	58.1 ± 1.4	63.1 ± 0.6

Tab. II. Percentile Conversion of Thiamine Hydrochloride to Thiochrome in an Alkaline Ferricyanide Solution Using Various Amounts of Methyl Alcohol in a Total Reaction Mixture of 8 ml. All Data Are the Average of Three or More Determinations.

ml CH_3OH	μg Thiamine Hydrochloride					
	0.1	0.2	0.4	1.0	200	1000
0	65.2	67.1	69.6	69.1	60.6	65.2
1	90.7	89.2	85.3	81.3	78.6	77.5
2	92.3	94.4	94.0	87.8	84.0	80.3
3	93.1	97.1	96.1	86.7	79.3	75.4

the effect is small and possibly can be ascribed to incomplete extraction into the isobutanol phase. In the presence of CH_3OH, the recovery of thiochrome from the reaction mixture is complete.

Results and Discussion. Theoretically, assuming 100% conversion of thiamine to thiochrome, 1μg of thiamine hydrochloride should produce 0·778μg of thiochrome. Preliminary series containing 0·1 to 0·4μg of thiamine and thiochrome respectively were run using 2 ml of CH_3OH in the reaction mixture. In this low concentration range the yield of thiochrome was not 100%, but a conversion of over 90% was indicated. Optimal conditions for maximum conversion were then determined by varying the amount of CH_3OH from 0 to 3 ml and also the range of thiamine concentration was increased. The results are given in Table II.

These data indicate that in both the low concentration range (0·1 to 1·0 μg/8 ml of reaction mixture) and the higher concentration range (200 and 1000μg/8 ml reaction mixture) there is a general increase in the percent conversion when the amount of CH_3OH is increased from 0 to 2 ml. In the lower range (using 2 ml of CH_3OH) the average maximum conversion is greater than 90% confirming JANSEN's[3] original results, while in the higher range the average conversion is still better than 80%. When CH_3OH is omitted from the reaction mixture there ist a 60–70% conversion in both the high and low ranges, confirming the results of CONNOR and STRAUB[7], EGANA and MEIKLEJOHN[8], and FERREBEE and CARDEN[9].

As ZIMA and WILLIAMS[4] pointed out, thiamine exists in solution in a state of equilibrium between the ammonium and the thiol type molecule. The equilibrium shifts to the ammonium type in an acid medium, while alkaline solutions favor the thiol type. Alkaline oxidation as used in this procedure could result in two types of reactions:

(a) An oxidative conversion of thiamine to thiochrome (SYKES and TODD[5]).

(b) An oxidative dimerization of the thiamine molecule to a non-fluorescent disulfide type molecule. Subsequently thiaminedisulfide could, under the conditions provided by the reaction mixture, possibly decompose to yield additional thiochrome (ZIMA and WILLIAMS)[4] and presumably thiamine thiazolone (SYKES and TODD)[5].

The data could be interpreted to indicate that in the absence of CH_3OH, part of the thiamine would follow the bimolecular type reaction. The presence of CH_3OH in the alkaline reaction mixture would change conditions to such an extent that more of the thiamine follows a monomolecular reaction type and is converted directly to thiochrome. Thus in the lower concentration range, the presence of CH_3OH (25% vol/vol) results in over 90% of the thiamine being oxidized to thiochrome according to the first reaction type. At higher concentrations, which would favor a bimolecular type reaction, the second reaction seems to become increasingly important.

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Zusammenfassung

Der Einfluss verschiedener Methylalkoholkonzentrationen auf die Thiamin-Thiochrom-Umsetzung im alkalischen Milieu mit 0·11% $K_3Fe(CN)_6$ wurde verfolgt. Im Reaktionsgemisch ohne CH_3OH wird im untersuchten Konzentrationsbereich (0·1 bis 1000μg in 8 ml Reaktionsgemisch) ungefähr 65% des Thiamins in Thiochrom umgewandelt. Im Reaktionsgemisch mit 2 ml CH_3OH in 8 ml wird der Umsatz über 90% für Thiaminkonzentrationen unter 1 μg und über 80% im Konzentrationsbereich von 1 bis 1000 μg Thiamin in 8 ml Reaktionsgemisch gesteigert.

Occurrence of Soluble Pigments in the Genus *Bacillus*

Pigmentation among species of the bacterial genus *Bacillus* is reported to be rare[1]. A few members of the genus do form pigmented colonies when cultured on agar media under certain conditions[2]. Little information has been published relative to the production of soluble pigments by the organisms grown in broth cultures. The above references do not describe pigmentation in broth by any members of the genus. However, *Bacillus anthracis* has been reported to produce a red[3] or purple-brown pigment[4] when cultured in liquid media. The present investigation was carried out to determine to what extent the common members of the genus *Bacillus* produced soluble pigments in broth cultures.

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