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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[2] G. S. WILSON and A. A. MILES, *Topley and Wilson's Principles of Bacteriology and Immunity*, 4th Ed. (Williams and Wilkins, Baltimore 1955), p. 952.

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The cultures employed were obtained from the collection of Dr. K. L. BURDON of this department. Species identification of the organisms had been carried out by accepted methods[5]. The bacteria were cultured in the chemically defined, liquid medium of THORNE *et al.*[6] containing 0·7% sodium bicarbonate. Inoculations were made from vegetative cell suspensions into 25 ml of medium contained in 250 ml flasks. The cultures were tightly closed with rubber stoppers, and incubated at 37°C for 24 h on a rotary shaker.

For visual determination of soluble pigment production, cells were removed from the cultures by centrifugation. The clear supernatant broth then was adjusted to pH 7·5 with 0·1 N NaOH, and compared to a blank of uninoculated broth treated in the same fashion. Acid sensitivity of the pigments was determined by adding 1 N HCl to the broth. Fluorescence was detected by examining the culture supernates under an ultraviolet lamp emitting its maximum light at 2,750 Å. In certain cases the absorption spectra of the pigments were determined in a spectrophotometer.

As can be seen from the Table cultures belonging in two of the six species examined (*B. anthracis* and *B. cereus*) produced visible pigments. The pigment of *B. cereus* was associated with fluorescence of the culture filtrate, while that of *B. anthracis* was sensitive to acid. The characteristic pink pigment of the latter organism was evident only under alkaline conditions; the culture filtrate became yellow when acidified. Spectrophotometric examination in the visible range demonstrated a broad absorption spectrum for the anthrax pigment with a single peak at 490 mμ. The yellow-brown pigment of *B. cereus* had no characteristic absorption spectrum in the visible range, but in the ultraviolet region the material evidenced a single peak at 350 mμ. The pigment was not sensitive to acid.

Since preliminary investigations showed that *B. anthracis* and *B. cereus* produced soluble pigments, several strains of these species were examined to determine whether the phenomena were characteristic of the species. The anthrax specimens consisted of both virulent and avirulent organisms. The Table shows that 15 of the 20 strains examined produced pigment. All of the *B. cereus* strains employed demostrated both the yellow-brown pigment and fluorescence.

JOHNSTONE and FISHBEIN[7] have suggested that florescent pigments might be used to distinguish species of *Azobacter*, and BRISOU[8] has made important use of pigmentation in the classification of chromogenic Gram negative bacteria, including the genera *Achromobacter*, *Flavobacterium*, *Pseudomonas*, *Serratia*, and *Xanthomonas*. Our data indicate that the absence or occurrence of soluble pigments in broth cultures of *Bacillus* species might be employed as an adjunct to other available methods for their classification. Of the six species examined *B. anthracis* was the only one producing a pH-sensitive, pink pigment, and *B. cereus* was the only one in which a yellow-brown pigment and fluorescence occurred. However, since not all anthrax strains produced the pigment, the character could be useful only in those strains in which it appeared. Absence of pigmentation would not mean the organism was not *B. anthracis*. In addition it should be emphasized that the pigmentation reported occurs only under the growth conditions specified. Since many factors such as incubation temperature, media composition, pH, *etc.* can affect pigmentation of bacteria, we believe the characteristic should be employed only as an ancillary criterion in classification. As a case in point it should be mentioned that although the presence of cellular pigments is one of the important criteria for classification of the genus *Serratia*[1, 2], colorless strains have been reported[9] as well as one strain which produced a soluble pigment[10].

Soluble Pigment Production in Broth Cultures by *Bacillus* Species

Species	No. of Strains	Pigment	% Positive	pH Sensitivity of Pigment	Fluorescence
B. anthracis	20	Pink	75	Yes	No
B. cereus	18	Yellow-Brown	100	No	Yes
B. licheniformis	2	None	0		No
B. megaterium	2	None	0		No
B. pumilis	2	None	0		No
B. subtilis	3	None	0		No

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Résumé

B. anthracis cultivé en bouillon dans certaines conditions précisées dans ce travail, produit un pigment rose sensible aux variations de pH. *B. cereus* produit de même, un pigment jaune-brun, fluorescent. Les pigments sont solubles et se retrouvent dans le filtrat. Quatre autres espèces de *Bacillus* ne produisent pas de pigments dans les mêmes conditions. La production de pigments en bouillons de culture pourrait être employée comme un critère additionnel pour la classification des souches *Bacillus*.

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Enzymatic Conversion of Metanephrine to Normetanephrine

Metanephrine (3-*O*-methylepinephrine) has been shown to be a major metabolic product of epinephrine and to occur normally in tissue and urine[1]. Since many methylated amines have been found to *N*-demethylate *in vitro*[2], the possibility that metanephrine might undergo such a reaction was examined.

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