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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Enzymatic N-Demethylation of Metanephrine

Microsomes obtained from 500 mg rabbit liver were incubated in air at 37°C with 3 μmoles metanephrine, 25 μmoles $MgCl_2$, 50 μmoles nicotinamide 50 μmoles neutralized semicarbazide hydrochloride, 0.25 ml pH 7.4 phosphate buffer (0.5 *M*), added cofactors and water to make a final volume of 3 ml. After 2 h, the incubated mixture was assayed for formaldehyde[4]

Additions	Formaldehyde Formed
	μmoles
Soluble fraction[a], TPN (0.5 μmole)	0.65
Soluble fraction[a]	0.20
Soluble fraction[a], TPN (0.5 μmole), microsomes omitted	0.00
TPN (0.5 μmole)	0.00
TPNH[b] (3 μmoles)	0.45
DPNH[b] (3 μmoles)	0.05

[a] Soluble fraction from 500 mg rabbit liver was dialyzed 20 h at 4°C against 0.01 *M* phosphate buffer pH 7.0.
[b] The cofactors were added in 6 divided portions over 90 min.

The enzymatic *N*-demethylation of metanephrine was studied by measuring the formaldehyde liberated after incubation of this amine with various cellular fractions of rabbit liver[3]. Incubation of microsomes with the soluble fraction of liver and triphosphopyridine nucleotide (TPN) resulted in the formation of formaldehyde (Table). When either the soluble fraction, TPN, or microsomes were omitted, little or no formaldehyde was formed. The soluble fraction and TPN could be partially replaced by reduced triphosphopyridine nucleotide (TPNH) but not reduced diphosphyopyridine nucleotide (DPNH).

Evidence for the identity of the *N*-demethylated product was obtained as follows: Microsomes and soluble supernatant fraction obtained from 5 g of rabbit liver were incubated at 37° with TPN, $MgCl_2$ and nicotinamide in pH 7·4 phosphate buffer. After 1 h the incubation mixture was adjusted to pH 9·5 and extracted twice with 5 vol of isoamyl alcohol. The isoamyl alcohol was re-extracted with 0·1N HCl and the acid extract was evaporated to dryness *in vacuo*. After taking up the residue in a small volume of ethanol, it was chromatographed on Whatman No. 1 paper, using isopropanol: ammonia (5%) 8:2 as the solvent system. The chromatogram was dried and a strip of an area corresponding to R_f 0·45 to 0·6 was cut out and eluted with methanol. The resulting extract contained a compound that gave the same R_f values in 3 solvent systems, color reactions and fluorescence spectrum as authentic normetanephrine[5]. These observations were taken as evidence that rabbit liver microsomes contained an enzyme requiring TPNH and other cofactors in the soluble fraction that could *N*-demethylate metanephrine to yield normetanephrine and formaldehyde. Guinea pig and rat liver also contain the metanephrine *N*-demethylating enzyme, but in smaller amounts.

The ability of metanephrine to form normetanephrine *in vivo* was examined after the administration of methoxy C^{14} metanephrine or H^3epinephrine to rats. No evidence for the presence of free and conjugated normetanephrine in the urine was found after the administration of these compounds.

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Zusammenfassung

In der Mikrosomen-Fraktion der Leber von Kaninchen, Ratte und Meerschweinchen wurde ein Ferment gefunden, welches Metanephrin zu Normetanephrin unter Formaldehydbildung N-demethylieren kann.

Nach Verabreichung von markiertem Epinephrin und Metanephrin konnten bei der Ratte keine Anhaltspunkte für die Ausscheidung von Normetanephrin gefunden werden.

Elevated α-2-Serum Proteins as a Possible Genetic Marker in Spontaneous Hereditary Diabetes mellitus of the Chinese Hamster (Cricetulus griseus)[1]

The occurrence of a spontaneous hereditary diabetes mellitus in the Chinese hamster has recently been reported[2]. This is a primary pancreatogenic condition, with degranulation, hydropic degeneration, and deficiency of β-cells. Degenerative changes in β-cells associated with development of clinical diabetes occur at an age specific for each subline of diabetic hamsters. The incidence of diabetes among progeny from diabetic parents depends upon the stage of inbreeding, diabetes occurring in animals which are 80% or more homozygous.

Electrophoretic patterns of serum proteins in families with a high incidence of spontaneous diabetes reveal α-2 levels to be two to three times the normal values (Table). In normal animals, the α-2 proteins generally are between 5–10% of the total serum proteins. When high incidence families were randomly hybridized by single or double crosses involving two or four grandparents, respectively, of diabetic background, normal values of α-2 are re-established.

Ejarque *et al*[3] have summarized previous work and have also reported slightly elevated α-2 serum proteins, and protein-bound carbohydrates in patients with diabetes. This elevation of α-2 values occurred generally when the diabetes was complicated by vascular and other secondary changes. In the Chinese hamster, however, α-2 serum proteins are increased, even prior to the onset of clinical

Percentage of the Total Serum Proteins in the α-2 Fractions in Various Families of the Chinese Hamster and Hybrids

% Protein α-2 Fraction	Families					Hybrids	
	JFY	VSY	BUY	HGY	ORY	(1)	(2)
0–5	1	0	0	0	1	7	0
6–10	1	0	6	4	3	12	8
11–15	3	6	5	0	1	0	0
16–20	2	2	7	0	0	1	4
21–25	1	2	8	2	0	1	1
26–30	0	0	6	1	0	0	0
31–35	0	0	1	0	0	0	0
Total	8	10	33	7	5	21	13

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