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.

J. Axelrod

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda (Maryland), June 10, 1960.

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

[1] This investigation was supported in part by research grants from the National Institutes of Health, USPHS #E1560 and #CY3335, the National Science Foundation (#6-9602), and the Damon Runyon Memorial Fund (#293).

[2] H. Meier and G. Yerganian, Proc. Soc. exp. Biol. Med. *100*, 810 (1959).

[3] P. Ejarque, A. Marble, and E. F. Tuller, Amer. J. Med. *27*, 221 (1959).

[4] Present address: Jackson Memorial Laboratory, Bar Harbor, Maine (USA).

diabetes, and microscopic evidence of intercapillary glomerulosclerosis. Breeding experiments are now in progress, using elevated α-2 serum proteins as a genetic marker in an attempt to increase the incidence of spontaneous diabetes. The extremely high levels of α-2 (up to 35%) observed in diabetic hamsters may be due either to an increase of normally existing α-2 proteins, or perhaps to new and different molecules which are a genetic reflection of the diabetic state. Chemical analysis of the α-2 globulin fractions in diabetic and non-diabetic hamsters would be required in order to distinguish between these two possibilities; such investigations are now in progress.

M. N. GREEN, G. YERGANIAN, and H. MEIER[4]

The Children's Cancer Research Foundation and the Department of Pathology, Harvard Medical School, at The Children's Hospital Medical Center, Boston (Massachusetts) May 10, 1960.

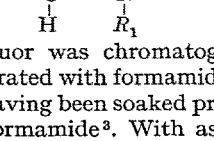
Zusammenfassung

Bei chinesischen Hamstern aus Inzuchten mit erblicher Zuckerkrankheit wurden erhöhte Serum-α-2-Eiweisswerte konstatiert. Die Erhöhung erfolgt vor Auftreten der Hyperglykämie oder Blutgefäss- und anderen sekundären pathologischen Veränderungen. Es wird gefolgert, dass die Eiweisserhöhung gen-bedingt ist und wahrscheinlich chemisch abnormales Eiweiss betrifft.

Isolation of *iso*-Butyropyrrothine along with Thiolutin and Aureothricin from a *Streptomyces* sp.

A new species of Streptomyces (*S. pimprina*) has been found to produce an undescribed antifungal antibiotic Hamycin of the polyene (heptaene) type[1]. Hamycin is present in the mycelium and the filtered broth was found to contain four antibiotics with antibacterial activity and, in addition, one colourless crystalline inactive compound.

The chloroform extract of the broth, on crystallization from benzene-methanol (60:40), gave a yellow crystalline compound (yield 50 mg/l), which was identified as the antibiotic Thiolutin[2] (I; $R = CH_3$; $R_1 = CH_3$) by comparison with an authentic sample (kindly supplied by Dr. A. C. FINLAY of Chas Pfizer & Co., Brooklyn, N. Y.).

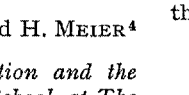


I

The mother liquor was chromatographed on paper, using benzene saturated with formamide as the developing phase, the paper having been soaked previously in acetone containing 20% formamide[3]. With ascending technique four yellow spots with the R_f (A) 0.0, (B) 0.4, (C) 0.71, and (D) 0.92 were obtained, which, when tested bioautographically with *Sarcina lutea* as the test organism, were found to be active.

The separation and the isolation of the antibiotics were done on cellulose powder, using formamide as the stationary phase and benzene saturated with formamide as the developing phase. Three moving bands together with one dark stationary band were observed. The three moving bands were collected separately and rechromatographed individually using a narrower column. The homogenity of each fraction was ascertained by paper chromatography.

The fast moving band (D, $R_f = 0.92$) was found to contain two components. One of them was an inactive colourless crystalline basic compound (D 1) which could be separated from the other by extracting a benzene solution of the mixture by dilute acid. Free base (yield, 20 mg/l) was crystallized from benzene into colourless prisms, m.p. 205°C [found, C 73.0, H 4.7, N 13.5%, M.W. 198 (Rast method)]. The other component was an orange-red active compound (D 2) which could be crystallized from benzene into orange-red plates, m. p. 228–229°C (found, C 47.4, H 4.8, N 10.9%) yield, 1 mg/l. This orange-red compound was identified as *iso*-butyropyrrothine[4]



(I; $R =$ –CH(CH$_3$)$_2$; $R_1 = CH_3$)

from its analysis and its identical IR, and UV spectra, identical antimicrobial spectrum and undepressed melting point with a 'synthetic' *iso*-butyropyrrothine prepared by the action of *iso*-butyrylchloride on pyrrothine hydrochloride[2] in pyridine. The 'synthetic' *iso*-butyropyrrothine, when purified through cellulose column gave m.p. 228–229°C. CELMER and SOLOMONS[5] mention its melting point as 222–228°C.

Natural *iso*-butyropyrrothine has an antimicrobial spectrum similar to Thiolutin. The *in vivo* inhibition concentration (in μg/ml) for various organisms is *Bacillus subtilis*, 1.25; *Sarcina lutea*, 0.45; *Staphylococcus aureus*, 1.0; *Candida albicans*, *Escherichia coli*, and *Salmonella paratyphi* were insensitive.

Another band (C, $R_f = 0.71$) gave a yellow-orange crystalline compound (yield 1 mg/l) which was identical to Aureothricin[6] (I; $R = CH_2–CH_3$; $R = CH_3$) in all obvious respects, including infra red, U. V. spectra, paper chromatographic behaviour and antimicrobial spectrum. 'Synthetic' Aureothricin was prepared by the method of CELMER and SOLOMONS[2] from pyrrothine hydrochloride and propionic anhydride.

The third moving band (B, $R_f = 0.4$) was found to be Thiolutin itself.

The stationary band (A, $R_f = 0.0$) at the top of the column was removed and extracted with methanol and the methanol extract was chromatographed on alumina (Activity III) in dry chloroform. A band which was eluted with chloroform-methanol (99:1) showed activity against *S. lutea* but the UV absorption spectrum was different from either Thiolutin group or Holomycin[3] (I; $R = CH_3$, $R_1 = H$). This fraction (A) was obtained in very small quantity and is not characterised as yet.

AKABORI and NAKAMURA[7] have obtained Thiolutin and Aureothricin along with 1-6-dihydroxyphenazine from a *Streptomyces* sp. To our knowledge, this is the first time that a *Streptomyces* sp. has been found to produce Thiolutin (acetopyrrothine), Aureothricin (propiopyrrothine),

Our thanks are due to Dr. M. J. THIRUMALACHAR for his interest in this work.

[1] Hindustan Antibiotics Limited, Indian Patent Appl. No. 71394.

[2] W. D. CELMER and I. A. SOLOMONS, J. Amer. chem. Soc. *77*, 2861 (1955).

[3] L. ETTLINGER, E. GÄUMANN, R. HÜTTER, W. KELLER-SCHIERLEIN, F. KRADOLFER, L. NEIPP, V. PRELOG, and H. ZAHNER, Helv. chim. Acta *42*, 563 (1959).

[4] For the nomenclature see foot note 5a of [2].

[5] W. D. CELMER and I. A. SOLOMONS, Antibiot. Ann. *1953–1954*, 622.

[6] H. NISHIMURA, T. KIMURA, and M. KUROYA, J. Antibiot., Tokyo [A] *6*, 57 (1953).

[7] H. AKABORI and M. NAKAMURA, J. Antibiot., Tokyo [A] *12*, 17 (1959).