and repetition at the level of the gene is no exception, particularly where it affects vital genes. But there are, of course, many influences on the rate of ageing of cells.

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Received December 21, 1971.

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Biosynthesis of Mescaline and N-Acetylmescaline by Mammalian Liver

WE have shown that mescaline, a well established hallucinogen found in the peyote cactus, can also be formed by mammalian liver from an appropriate precursor. An enzyme can be extracted from various mammalian tissues (by centrifugation at 100,000*g* for 1 h), including human brain and liver that transfers a methyl group from S-adenosyl[Me-^{14}C]methionine (^{14}C-SAM) to 4-O-methylated catecholamine metabolites to form corresponding 3,4-di-O-methylated compounds[1,2]. One of the substrates for this enzyme is iso-N-acetylmethoxytyramine (i-NAMT), which is converted to N-acetyl-3,4-dimethoxy-β-phenethylamine (NADMPEA). An enzyme in the same fraction of liver can transform 4-hydroxy-3,5-dimethoxy-β-phenethylamine (4-desmethylmescaline or 4-DMM) to mescaline (3,4,5-trimethoxy-β-phenethylamine), and N-acetyl-4-hydroxy-3,5-dimethoxyphenethylamine (N-acetyl-4-desmethyl-mescaline or NA-4-DMM) to N-acetylmescaline.

The supernatant fraction of rat liver obtained after centrifugation at 100,000*g* for 1 h (*p*H 9.0, 0.1 M Tris-HCl buffer) was used with incubation conditions as described in Table 1. Blanks consisted of samples less substrate, or with protein modified by heating. Reactions were terminated by the addition of 0.2 ml. of 3 N HCl for acidic and neutral metabolites and 0.2 ml. of 1 N NaOH for basic metabolites. The sample was extracted once with 6 ml. of toluene and isoamyl alcohol, 90 : 10 (v/v). The extract was evaporated to dryness with nitrogen and the residue redissolved in methanol with the appropriate reference compound, chromatographed on silica gel fibreglass strips (Gelman Instrument Co.) in benzene, heptane and diethylamine 10 : 5 : 1, and the radioactivity in the appropriate area was determined by liquid scintillation spectroscopy. The recovery of both products was better than 80%.

Table 1 shows that in the presence of substrate a radioactive product was formed which was extractable into the solvent and recovered after chromatography. When substrates were omitted, only a small amount of radioactivity was recovered. These results indicate that there is an enzyme present which catalyses the transfer of a ^{14}C-methyl group in significant amounts from ^{14}C-SAM to each of the substrates.

The radioactive product, presumably mescaline, formed after incubation of 4-DMM was further identified by chromatography in four solvent systems, by co-crystallization to constant specific activity and by derivatization to N-acetylmescaline, 2,6-dibromo-N-acetylmescaline and trimethoxybenzoic acid, which were further recrystallized without loss of molar specific activity. The radioactive product derived from incubations of NA-4-DMM was characterized by similar methods.

When the supernatant fraction was used in incubations with 4-DMM or NA-4-DMM, maximal activity was obtained at about *p*H 9 in the presence of Mg^{2+}. Biosynthesis of the mescalines is strongly inhibited by 10^{-3} M EDTA, and activity can be restored by the inclusion of 10^{-1} M $MgCl_2$ in an incubate containing 10^{-3} M EDTA (Table 1). The K_m against NA-4-DMM is around 10^{-4} M. The K_m against SAM (10^{-3} M NA-4-DMM) is about 10^{-5} M. The reaction maintains good linearity through a ten-fold range of protein concentrations for both 4-DMM and NA-4-DMM. These properties are similar to those for the enzyme involved in the formation of di-O-methylated catecholamine metabolites except that the K_m against i-NAMT is 6.9×10^{-4} M. The mescaline-forming enzyme is most active against substrates carrying the hydroxy substituent in the 4 position. There was relatively little activity against 3-DMM. In contrast, in the formation of dimethoxy compounds, the enzyme preferentially transfers a methyl group to the 3 position (our unpublished results). The presence of both a 3 and a 5-methoxy substituent on the favoured mescaline precursor may have a strong directional effect on the O-methylating enzyme, which overcomes expected steric hindrance toward methylation of the 4 hydroxy group. Alternatively, mescaline and N-acetyl-

Table 1 Rates of Formation of Mescaline and N-Acetylmescaline in Various Conditions

System	Substrate (M)	Product formed* (d.p.m./mg super. protein/h) Mescaline (from 4-DMM)	N-Acetylmescaline (from NA-4-DMM)
I, Rat liver			
Boiled protein	1×10^{-3}	200	185
No substrate	0	325	280
Complete	1×10^{-4}	19,000	40,000
Complete	2×10^{-4}	—	57,500
Complete	3×10^{-4}	—	61,500
Complete	5×10^{-4}	—	70,100
Complete	1×10^{-3}	34,000	—
Complete	2×10^{-3}	36,400	—
Complete	5×10^{-4}	35,800	63,200
No Mg^{2+}, EDTA 10^{-3} M	5×10^{-4}	1,650	920
Mg^{2+} 10^{-1} M, EDTA 10^{-3} M	5×10^{-4}	36,200	70,600
1.0 mg protein	1×10^{-3}	32,800	—
2.0 mg protein	1×10^{-3}	34,000	—
5.0 mg protein	1×10^{-3}	31,200	—
0.65 mg protein	1×10^{-4}	—	25,500
1.3 mg protein	1×10^{-4}	—	29,300
2.6 mg protein	1×10^{-4}	—	31,500
^{14}C-SAM 4×10^{-6} M	1×10^{-3}	29,500	—
^{14}C-SAM 8×10^{-6} M	1×10^{-3}	43,600	—
^{14}C-SAM 2×10^{-5} M	1×10^{-3}	70,800	—
^{14}C-SAM 1.65×10^{-6} M	1×10^{-4}	—	17,800
^{14}C-SAM 3.3×10^{-6} M	1×10^{-4}	—	29,600
^{14}C-SAM 6.6×10^{-6} M	1×10^{-4}	—	48,500
^{14}C-SAM 1.65×10^{-5} M	1×10^{-4}	—	71,100
II, Human liver (12 h post-mortem)			
^{14}C-SAM 2×10^{-5} M	0	608	—
^{14}C-SAM 2×10^{-5} M	1×10^{-3}	3,990	—

Complete system: 2 mg protein from 100,000*g* for 1 h dialysed tissue supernatant, 200 μmol, Tris-HCl buffer, *p*H 9.0, 10 μmol $MgCl_2$, 4 mμmol ^{14}C-SAM (specific activity 52.3 mCi/mmol) and the concentration of 4-DMM or NA-4-DMM listed, in a total volume of 1 ml. Incubations at 37° C were carried out for 15 min. All preparations listed are complete except for departures noted. Human tissues were not dialysed. Results are corrected for low blank values obtained without substrate, but are not corrected for recovery.
* All data are rounded to 3 significant figures.

mescaline may be formed by an enzyme different from that involved in the formation of the dimethoxy compounds.

Neither the dimethoxy compounds nor mescaline seem to be formed through the action of any of the O-methyltransferases described by other investigators. Catechol-O-methyltransferase requires that substrates have an adjacent free hydroxy group[3]. Hydroxy-indole-O-methyltransferase is found only in mammalian pineal gland[4], and the best substrate for this enzyme, N-acetyl-serotonin, was not methylated in our system. Phenol-O-methyltransferase is found only in the particulate fraction, is inhibited by Mg^{2+}, is not inhibited by 10^{-3} M EDTA, and does not methylate 4-DMM (ref. 5). In addition, we have tested microsomal phenol-O-methyltransferase for activity against both phenol and i-NAMT. Only the former yielded a labelled product (anisole). Another O-methyltransferase, iodophenol-O-methyltransferase, has a low *p*H optimum, approximately *p*H 6 (ref. 6), and does not require Mg^{2+}. In contrast, the formation of di-O-methylated compounds proceeds more than 1,000 times as rapidly at *p*H 9 as at *p*H 6 and the mescalines are formed more than ten times as rapidly at the higher *p*H.

Some of the substrates involved in the formation of the dimethoxy compounds are known to be formed by mammalian tissues[7,8]. Neither 4-DMM nor NA-4-DMM, however, are known in mammalian tissues and no metabolic route for their formation has been established in mammals. A compound, 3-methoxy-4,5-dihydroxyphenethylamine, which could conceivably serve as a precursor of 4-DMM has been reported to be formed by rat and rabbit liver from 3-methoxytyramine. The 3-methoxy-4,5-dihydroxy product, however, was not identified definitely[9]. Also the formation of 5-hydroxy derivatives of other 3-O-methylated catecholamine metabolites has been shown[10]. These might serve as a metabolic link between catecholamines and mescaline. It is not known, however, whether any of these reactions have physiological significance or can take place *in vivo*.

This work was supported by US Public Health Service grants and a Research Scientist Award to A. J. F. We thank Mary Armour and Ingrid Sherman for technical assistance.

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Received November 29, 1971; revised January 24, 1972.

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Difference in Chromosome Lengths between Male and Female Sheep

A STUDY of the lengths of the chromosomes in sheep and goats[1] suggested incidentally that there are small differences between the sexes in the lengths of some chromosome pairs. Here I report the measurement of thirty chromosome spreads from thirteen genetically normal male sheep and thirty-one spreads from thirteen normal ewes, showing differences between the sexes in two pairs of large autosomes which were longer in the males than in the females. Nine spreads were examined from an intersex sheep, a genetic female which had well-developed testes and masculinized genitalia. The lengths of some of the chromosomes in her karyotype resembled those of normal males. The observations may indicate some autosomal control of the differentiation of sex.

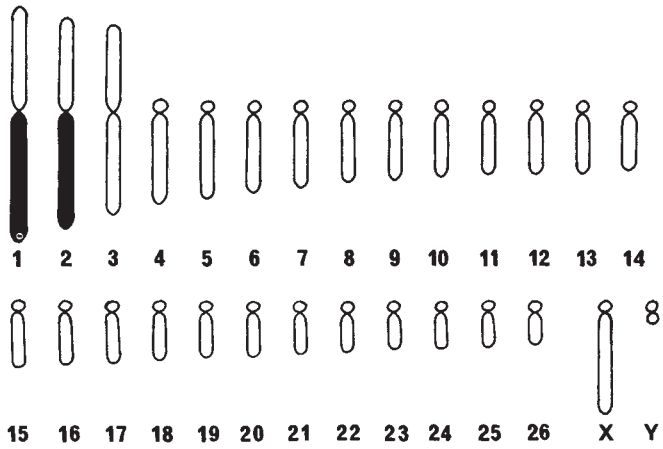

Fig. 1 The twenty-six autosomes of the haploid complement and the sex chromosome pair of the sheep (2n=54). The diploid complement has the fundamental number of sixty arm-pairs.

The preparation of chromosome spreads from blood lymphocytes and the measurements of length were carried out as previously described[1]. The karyotype was treated as sixty arm-pairs and each pair was calculated as its percentage of the total length of chromatin in the spread. The divisor was adjusted in each case so that the male and female karyotypes were comparable. This was done by subtracting the length of the *Y* chromosome from the total length of chromatin in a male spread; in the females the divisor was the total length of chromatin without the shorter *X* chromosome. Although these measurements are not in absolute units they provide a useful comparison of the two groups. Also each chromosome of the haploid complement was expressed as a ratio, using the single *X* chromosome of the males or the longest *X* chromosome of the females as a divisor.

The chromosomes which showed differences in length are identified in Fig. 1, and the observations are summarized in Tables 1 and 2. The measurements, expressed as percentages, compared each arm-pair with the total length of chromatin in the complement. They showed that the long arms of the first and second metacentric pairs and the fourth pair, which is acrocentric, were longer in the males than in the females. The difference between the males and females in the long arms of the first pair was 0.25% ($0.01 > P > 0.002$); in the long arms of the second pair the difference was 0.19% ($0.02 > P > 0.01$) and the difference between the sexes in the fourth pair of autosomes was 0.09% ($0.05 > d > 0.01$). Six pairs of short acrocentrics were slightly longer in the females than in the males ($0.05 > P > 0.02$). In the nature of percentages, however, surplus length in one part of the range must result in a shortage at another; it may be difficult to judge whether this tendency is the result of real differences in length, or due to a bias introduced by the use of percentages. In the alternative examination of the measurements, it was assumed that the *X* chromosome of the males and the longest *X* chromosome of the females are of equal length and ratios were calculated of each autosomal arm to the *X* chromosome. It was found that the length differences between males and females persisted in the long arms of the first and second pairs of metacentrics, but that the differences among the acrocentrics were no longer significant. Thus both methods of assessment record a greater length in the long arms of the first and second pairs of metacentric chromosomes in the males. These observations are consistent with the presence of male-determining genes on the long arms of the first two pairs of autosomes. (These arms