Table 1. Enzymatic *N*-methylation of tryptamine in rat and human brain. Brain supernatant fractions incubated with adenosyl[methyl-^{14}C]methionine, with and without tryptamine or *N*-methyltryptamine, were extracted and evaporated as described in the text.

Treatment		Substrate added	Enzymatic activity	
Brain supernatant	Organic extract		Disintegrations per minute per 90 minutes of incubation	Picomoles per gram of tissue per hour
		Rat brain		
None	None	None	3020	
None	Evaporated	None	199	
None	Evaporated	Tryptamine	341	40
Dialyzed	Evaporated	None	169	
Dialyzed	Evaporated	Tryptamine	1350	350
Dialyzed	Evaporated	*N*-Methyltryptamine	995	250
		Human brain		
Dialyzed	Evaporated	None	132	
Dialyzed	Evaporated	Tryptamine	1650	450
Dialyzed	Evaporated	*N*-Methyltryptamine	1320	360

ethanol and 10 ml of phosphor, and the radioactivity was counted. The addition of tryptamine to the incubation mixture resulted in the formation of a radioactive product (Table 1). Heated enzyme was not active. For identification of the product, larger amounts of brain extracts and substrates were incubated and extracted as above. The solvent extract was evaporated at room temperature under nitrogen atmosphere and chromatographed in systems A, B, C, D, and E (*10*). Radioactive peaks isographic with authentic *N*-methyltryptamine and smaller peaks isographic with authentic dimethyltryptamine were found in all of the solvents used. The conversion of *N*-methyltryptamine to dimethyltryptamine was also confirmed with the use of *N*-methyltryptamine as a substrate. The radioactive product formed was identified as dimethyltryptamine in the same solvent systems as above. Human brain biopsy specimens from temporal lobe, obtained from neurosurgical operations, were also assayed for their enzymatic *N*-methylating activity. All human brain samples were capable of *N*-methylating tryptamine to *N*-methyltryptamine.

The normal occurrence of tryptamine in the brain, as well as its capacity to form a psychotomimetic *N*-methylated metabolite has implications to pharmacology, psychiatry, and biochemistry. The presence of an inhibitor of the *N*-methylating enzyme suggests that its activity might be regulated by endogenous compounds in the brain. The nature of the normally occurring inhibitor of the methylating enzyme has not been established. However, serotonin, catecholamines, and histamine could inhibit the tryptamine methylation since they are substrates for the nonspecific methylating enzyme (*2*) as well as other *S*-adenosylmethionine–requiring enzymes (*11, 12*). These biogenic amines are highly localized in different regions of the brain (*15*). In addition, *S*-adenosylhomocysteine, a compound normally occurring in the brain, has recently been reported to produce inhibition of several methyltransferases (*16*). Thus it is possible that changes in levels of serotonin, catecholamines, histamine, or *S*-adenosylmethionine might affect the *N*-methylation of tryptamine in specific brain areas.

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13 December 1971

1-Methyladenine Biosynthesis in Starfish Ovary: Action of Gonad-Stimulating Hormone in Methylation

Abstract. *Gonad-stimulating substance, a hormonal peptide released from the nervous system of starfishes, acts on the ovary to produce 1-methyladenine, an inducer of oocyte maturation. Addition of methionine to the incubation mixture of ovarian fragments and gonad-stimulating substance enhanced the production of 1-methyladenine, whereas ethionine inhibited it. Incubation of ovarian tissue with methionine alone failed to produce 1-methyladenine. Use of a radioactive label showed that methionine is a methyl donor in the biosynthesis of 1-methyladenine, suggesting that gonad-stimulating substance is involved in the methylation process.*

Oocyte maturation and spawning in the starfish are triggered by a hormonal peptide, gonad-stimulating substance (GSS), which is released from nervous tissue (*1*). This peptide consists of about 22 amino acids and has a molecular weight of 2100 (*2*). The action of GSS is indirect (*3*); it acts on the ovary to produce maturation-inducing substance (MIS), which is a direct inducer of oocyte maturation and spawning in starfishes. The substance MIS has been isolated and identified as 1-methyladenine (*4*). Thus the role of GSS in such reproductive phenomena seems to be to synthesize 1-methyladenine, which is produced by the follicles around the oocytes (*5*). Since methionine—in its active form, *S*-adenosylmethionine (*6*)—donates the methyl

group in many biological methylations, the effect of methionine was investigated to determine whether this substance is also a methyl donor in the synthesis of 1-methyladenine in the starfish ovary.

Isolated ovarian fragments of *Asterina pectinifera* in artificial sea water (*7*) (250 mg of wet ovary per milliliter) were incubated with L-methionine (*8*) in various concentrations (10^{-9} to $10^{-2}M$) for 1 hour at 25°C. A volume of GSS-water (*9*) equal to one-tenth of the incubation volume was added (the GSS-water contained 2 mg of dry nerve per milliliter), and incubation was continued for an additional hour. After incubation, the mixture was centrifuged at 3000 rev/min for 15 minutes, and the supernatant was assayed for maturation-inducing activity. For bioassay, the supernatant was diluted serially with sea water, and 150 to 250 isolated oocytes were placed in 0.5 ml of test solution. After 1 hour the rate of breakdown of the germinal vesicles was checked. Various concentrations (10^{-9} to $10^{-6}M$) of authentic 1-methyladenine (Sigma) in sea water were used as standards to check the reactivity of the oocytes, and the maturation-inducing activity of the test solutions was expressed as the amount of 1-methyladenine that had equivalent activity. The addition of methionine enhanced production of MIS in isolated ovary in the presence of GSS (Fig 1).

The next experiment tested whether L-ethionine (*8*) is a competitive inhibitor of methionine in the production of MIS in the presence of GSS. Six incubations (A to F) of ovarian fragments (200 mg/ml) were done for 1 hour at 20°C. Incubation media were A, sea water; B, sea water containing $10^{-3}M$ methionine; C, sea water; D, sea water containing $10^{-3}M$ methionine; E, sea water containing $10^{-3}M$ ethionine; and F, sea water containing $10^{-3}M$ methionine and $10^{-3}M$ ethionine. Then 0.1 ml of deionized water was added to incubations A and B, and 0.1 ml of GSS-water (2 mg of dry nerve per milliliter) was added to incubations C to F; then incubation was continued for an additional hour. The mixtures were then centrifuged, and the supernatants were assayed as in the previous experiment.

Ovarian fragments did not produce MIS (that is, the amount of MIS was not detectable) when they were incubated in sea water alone or in the presence of methionine. In contrast, fragments produced MIS in the presence of GSS; MIS activity per milliliter of supernatant was equivalent to that of 0.041 ± 0.008 μg of 1-methyladenine (mean ± S.E.; five experiments). Addition of methionine promoted the production of MIS in the presence of GSS; MIS activity was equivalent to that of 0.076 ± 0.028 μg of 1-methyladenine, and was 85 percent greater than activity when methionine had not been present. In contrast, ethionine severely inhibited (by 85 percent) the GSS-stimulated MIS production; MIS activity was equivalent to that of 0.006 ± 0.001 μg of 1-methyladenine. When methionine and ethionine were present at equal concentrations, methionine reversed this inhibitory action of ethionine; MIS activity was equivalent to that of 0.059 ± 0.021 μg of 1-methyladenine.

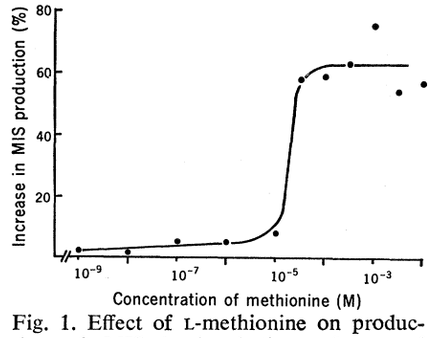

Fig. 1. Effect of L-methionine on production of MIS in incubation mixture of ovary and GSS of *A. pectinifera*. Final concentration of GSS in reaction mixture was 182 μg of dry nerve per milliliter.

The results above suggest that methionine may be a methyl donor in the GSS-stimulated synthesis of 1-methyladenine. Lyophilized [methyl-^{14}C]L-methionine (*10*) was dissolved in deionized water to give a concentration of $10^{-3}M$, and 0.2 ml (10.8 μc) of this was added to centrifuge tubes containing 500 mg of ovarian fragments in 1.6 ml of artificial sea water (*p*H 8.5). After incubation for 30 minutes at 25°C, 0.2 ml of GSS-water (2 mg of dry nerve per milliliter) was added to the incubation mixture, which was then incubated for an additional 2 hours (the final concentration of methionine was $10^{-4}M$). The supernatant was collected by centrifugation at 3000 rev/min for 15 minutes and frozen until used.

Gel filtration was performed with 1.5 ml of this supernatant on a Sephadex G-15 column (1.41 by 39 cm); the column had been equilibrated with artificial sea water (*p*H 8.5), which was used as eluant (25°C). The flow rate was 36 ml/hour and the fraction size was 2 ml. A 1-ml sample was taken from each fraction and mixed with 10 ml of scintillation liquid (*11*), and its radioactivity was determined with a Beckman DPM-100 liquid scintillation system. There was a distinct peak of radioactivity at the site of elution of authentic 1-methyladenine from the same column.

In another experiment 1.35 ml of the supernatant was mixed with 0.15 ml of $10^{-3}M$ 1-methyladenine and fractionated on the same column under the same conditions. In this case the fraction size was 3 ml. For each fraction, absorbance at 260 nm was first determined, then a sample of 1 ml was

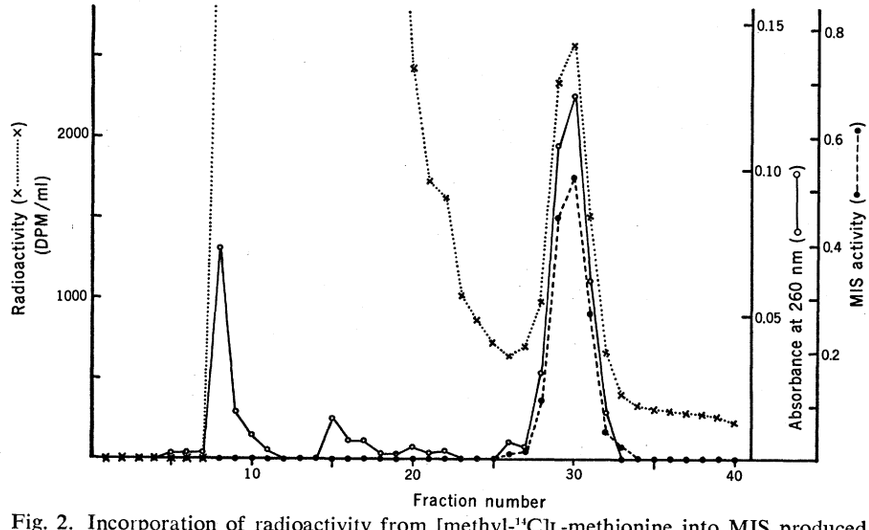

Fig. 2. Incorporation of radioactivity from [methyl-^{14}C]L-methionine into MIS produced in the ovary of *A. pectinifera* in the presence of GSS (*DPM*, disintegrations per minute). When L-methionine alone was applied on the same column, it was eluted in fractions 14 to 16 with a peak at fraction 15, as detected by both radioactivity and ninhydrin reaction. In the bioassay, MIS activity is expressed as the amount of 1-methyladenine that had equivalent activity.

taken for a measurement of radioactivity, and another of 0.1 ml was taken for bioassay. Results are shown in Fig. 2. It is probable that methionine is a methyl donor in 1-methyladenine production, since the elution pattern of the radioactivity was in good agreement with those of the biological activity and absorbance at 260 nm of 1-methyladenine. The amount of 1-methyladenine was very reliably determined by the bioassay; this assay indicated that the MIS produced in the ovary in the presence of GSS and methionine was 1-methyladenine.

The remaining samples of the biologically active fractions were pooled, concentrated to about 1 ml, and applied to a Sephadex G-15 column (1.37 by 39 cm) equilibrated with 0.2*M* pyridine acetate buffer (*p*H 8.4), which was used as eluant. The flow rate was 40 ml/hour and the fraction size was 3 ml. The remainders of fractions with high radioactivity (a 0.5-ml sample from each fraction had been used to determine the radioactivity) were pooled, concentrated to dryness, dissolved in a small amount of deionized water, and applied to a thin-layer chromatographic plate of microcrystalline cellulose (*12*). The plate was developed with isopropanol : hydrochloric acid : water (65 : 16.7 : 18.3, by volume). A single spot was detected by an ultraviolet light lamp. Both the radioactivity, determined by a gas-flow thin-layer chromatography scanner (*13*), and the maturation-inducing activity were confined to this spot. From these experiments it is clear that the methyl radical of L-methionine is incorporated into 1-methyladenine synthesized in the starfish ovary under the influence of the gonad-stimulating hormonal peptide, GSS.

The precise biochemical pathway of 1-methyladenine synthesis in the starfish ovary is not known. Although 1-methyladenine is a minor base in nucleic acids, especially some transfer RNA's, the 1-methyladenine produced under the influence of GSS is not a breakdown product of nucleic acids but is newly synthesized (*14*). The fact that isolated ovary can produce 1-methyladenine from 1-methyladenosine by the action of 1-methyladenosine ribohydrolase (*15*) suggests that 1-methyladenosine is an immediate precursor of 1-methyladenine. 1-Methyladenosine monophosphate (1-methyl AMP) also served as a precursor of 1-methyladenine in isolated ovary (*14*). However, the production of 1-methyladenine from either 1-methyladenosine or 1-methyl AMP is unaffected by the presence of GSS.

On the other hand, the results we report clearly show that methionine enhances the production of 1-methyladenine in the presence of GSS. In the absence of GSS, production of 1-methyladenine is not detectable. These facts suggest that the hormonal peptide GSS, which is released from nervous tissue, stimulates methylation of the N-1 position of the purine nucleus of a precursor of 1-methyladenine. Neither the enzyme involved in this methylation nor the acceptor of the methyl radical is yet known.

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Galactose Utilization in Galactosemia

Abstract. *Cultures of human galactosemic fibroblasts without detectable transferase activity were able to convert [1-^{14}C]galactose to $^{14}CO_2$ to the same extent as normal cells, but did so at a significantly slower rate. The utilization of galactose in both normal and galactosemic cells was strongly inhibited by glucose at physiologic concentrations.*

The defect in galactosemia is generally accepted as an absence of activity of the enzyme galactose-1-phosphate uridyltransferase (transferase) (*1*). More recently it has been shown that a protein, immunologically identical with active transferase, is produced in cells derived from galactosemic patients; this finding suggests that the basic defect is a mutation in the structural gene which then results in an inactive enzyme (*2*).

A number of investigators have studied galactose metabolism in galactosemia both in vivo and in vitro (*3, 4*), and some rapid screening procedures for transferase activity deficiency have been based on the demonstration in vitro of little or no $^{14}CO_2$ production from [1-^{14}C]galactose by fibroblasts, leukocytes, or whole blood of galactosemics (*5, 6*). Other reports, however, have shown that [1-^{14}C]galactose can be converted to $^{14}CO_2$ in vivo by galactosemic patients and that the ability to metabolize galactose is greatly stimulated by progesterone (*7*). In addition, the presence of low levels of transferase activity in some galactosemic fibroblasts has been described (*8*). We now report that fibroblast cultures derived from a galactosemic patient which have no detectable transferase activity when assayed with a radioactive substrate (*9*) can convert [1-^{14}C]galactose to $^{14}CO_2$, and that glucose inhibits galactose utilization in this system.

A diploid cell line, CCL-72, from a human galactosemic patient was obtained from the American Type Culture Collection, Rockville, Maryland, and the normal human diploid cell line, WI-38, was obtained from HEM Research, Inc., Rockville, Maryland. Cells were grown in monolayer culture to con-