follows an inherent trend. It has earlier been assumed[9,12] that the differentiation of the vaginal müllerian epithelium is induced by the sinus epithelium. The results from this investigation suggest that this is unlikely. If the sinus epithelium acted as an inductor, the induction should take place immediately the tips of the müllerian ducts reach the dorsal wall of the urogenital sinus, and the effect of this induction would not be evident until 7 or 8 days later. Instead, it seems more reasonable to regard the mesenchyme-epithelial relationship as an important factor in the differentiation process of the müllerian vaginal epithelium, this agreeing with the results from several other tissues[5]. The failure so far to get a normal differentiation of the müllerian epithelium by in vitro experiments has seriously hampered further analysis of our system[13].

Zusammenfassung. Die Resultate dieser Untersuchung unterstützen die Auffassung, dass das Epithel im kranialen Teil der Vagina ein Derivat des Müllerschen Epithels ist. Seine Differenzierung erscheint durch Zusammenwirken von Mesenchym und Epithel verursacht.

J.-G. FORSBERG and KRISTINA NORELL

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On the Incorporation of Uridilate into Cerebro-Cortex Ribonucleic Acid

The synthesis of ribonucleic acid (RNA) in nerve cells presents points of particular interest. It is known that unusual amounts of RNA are present in neurons where they could be involved in the storage mechanism of memory[1]. Moreover, an alteration of the base ratio composition of nuclear and cytoplasmic RNA has been registered in those neurons directly involved in establishing particular patterns of sensory and motor abilities[2,3]. The presence has also been reported, in both grey and white matter, of a DNA-directed RNA-polymerase[4]. In this note the incorporation of uridine triphosphate (UTP) into a RNA-like polymer is described. The incorporation is catalysed by a subcellular fraction prepared from the cortex of calf brain.

Preparation of the enzyme. The cortex layer was scraped from the surface of meninges-free calf brain and the tissue was homogenized with an equal volume of sucrose buffered solution (0.25 *M* sucrose, 0.15 *M* *tris*-HCl, 0.05 *M* 2-mercaptoethanol, 0.1 *M* versene, pH 7.5). After centrifugation at 20,000 *g* for 15 min the supernatant was diluted with an equal volume of *tris*-HCl 0.01 *M*, pH 7.5, containing 0.05 *M* 2-mercaptoethanol and centrifuged for 120 min at 105,000 *g*. The sediment was then suspended in 0.4 *M* *tris*-HCl, pH 7.5, containing 0.05 *M* 2-mercaptoethanol and used as the source of enzyme. This fraction contained no DNA and a RNA/protein ratio of 0.06. All the operations were carried out at 0–2 °C. DNA was determined by the BURTON procedure[5], RNA by a modification of the orcinol procedure[6], and the protein by the LOWRY method[7].

Preparation of labelled UTP. Uridine triphosphate labelled with ^{32}P in the α-phosphate was prepared as follows. *Escherichia coli* was grown on $H_3{}^{32}PO_4$, the nucleates were extracted with phenol, the DNA digested with deoxy-ribonuclease, the RNA hydrolysed with snake venom diesterase, the labelled 5′-mononucleotides were separated by Dowex I formate chromatography and phosphorylated to triphosphates with *E. coli* kinases, and finally the triphosphates were purified by column chromatography[8]. Uridine 5′-triphosphate-2-C^{14} was obtained from Schwarz BioResearch Inc., USA.

Experimental procedure. The incubation mixture contained the following components in 11 ml: 880 μmoles *tris*-HCl pH 7.5, 220 μmoles 2-mercaptoethanol, 110 μmoles potassium 3-phosphoglycerate, 55 μmoles $MgCl_2$, 5.5 μmoles UTP^{32} (specific activity $11 \cdot 10^6$ counts/min per μmole) or UTP 2-C^{14} (specific activity $4.1 \cdot 10^6$ counts/min per μmole), 5.5 mg muscle glycolytic enzymes[9], and 68 mg protein (enzyme).

All the incubations were carried out at 38 °C for 20 min. At the end of the incubation, the mixture was brought with perchloric acid (PCA) to a final concentration of 0.4 *M* and to a final volume of 35 ml. After 5 min standing in an ice bath, the mixture was centrifuged and the precipitate washed 4 times with 35 ml cold PCA 0.4 *M*. The nucleic acids were extracted from the precipitate at pH 7.4 with 4.5 ml hot 2 *M* NaCl in 2 successive 30 min extractions. The nucleates were precipitated from the extract with 2 volumes of absolute ethanol at − 15 °C, allowing 30 min before centrifugation. The precipitate was redissolved in 2 ml 2% (w/v) potassium acetate and the alcohol precipitation was repeated. After a washing with ethanol 75% the precipitate was dissolved in 1 ml potassium hydroxide (KOH) 0.3 *M* and let stand for 18 h at 37 °C. The hydrolysed mixture was then neutralized with PCA and the insoluble perchlorate was centrifuged out.

Aliquots of the supernatant were used from chromatography and counting. The ribonucleotides were separated by column chromatography on Dowex 1 formate[10]. A two-dimensional thin layer chromatography was also carried out using cellulose 300 MN as support and isobutyric

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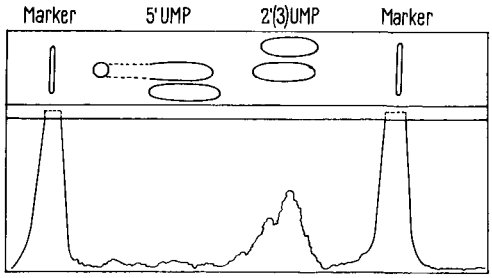
acid-ammonia[11] and isopropanol-water-ammonia[12] as developing systems. For each solvent the running time was 3 h. The spots localized by UV-illuminations were scraped off, suspended in scintillation fluid and counted. The scintillation fluid had the following components: toluene 500 ml, dioxane 500 ml, methanol 300 ml, PPO 6.0 g, POPOP 0.150 g, naphthalene 100 g, plus 4% Cab-o-sil.

Nucleotide composition

Substance	By UV	By ^{32}P distribution			
	Column	Column		Thin layer chromatography	
	%	Counts per min	% of total	Counts per min	% of total
2′(3′)-CMP	33.3	159	7.0	15.5	12
2′(3′)-GMP	30.8	200	8.8	11.5	9
2′(3′)-UMP	17.2	1735	75.7	92.0	73
2′(3′)-AMP	18.7	194	8.5	7.5	6
Recovery		2288	92.5	126.5	91

CMP = cytidine-monophosphoric acid; GMP = guanosine-monophosphoric acid; UMP = uridine-monophosphoric acid; AMP = adenosine-monophosphoric acid.



Radiochromatogram of the UMP fraction. Chromatography on Whatman No. 3 mm paper. Developing solvent, 6:3:1 absolute ethanol-2% (w/v) H_3BO_3-NH_4OH (density 0.9)[10]. Bottom: reproduction of a radiochromatogram of 2′(3′)-UMP; full-scale deflection = 1000 counts per min. Top: reproduction of the separation of two authentic samples of 5′-UMP and 2′(3′)-UMP.

In addition, the uridylate fractions were chromatographed on paper with 0.5 μmoles authentic uridine-5′-monophosphoric acid (5′-UMP) using the borate system[13], which separates 2′(3′)-nucleotides from 5′-nucleotides.

Results and conclusions. The ^{32}P distribution in the alkaline hydrolysate of the labelled RNA extracted is shown in the Table. 75% of the ^{32}P counts were found in 2′(3′)-UMP. This is confirmed by the results of the paper chromatography shown in the Figure. The incorporation is mostly not terminal. In fact, when UTP labelled in the pyrimidine ring was used as precursor, only 29% of the radioactivity was found associated with the nucleoside. It is concluded that a subcellular fraction which apparently contains no DNA and probably derives from the microsomes is able to incorporate uridylate into RNA when UTP is the precursor. The incorporation is largely not terminal and not random. In some aspects, the system described here is similar to the pigeon liver microsome system of STRAUS and GOLDWASSER[13]. It would be interesting to determine whether the peculiarity of the product is due to a specific mechanism at work in vivo or to an artefact due to the extraction of the enzymatic activity from the cell.

Riassunto. Una frazione subcellulare preparata dalla corteccia cerebrale del vitello è capace di catalizzare l'incorporazione di uridilato nell'acido ribonucleico (RNA) quando UTP è usato come precursore. L'acido ribonucleico sintetizzato risulta costituito per il 75% di residui uridilici di cui soltanto il 29% risulta terminale.

A. ORENGO[14]

Houston State Psychiatric Institute, Houston (Texas, USA) and Stazione Zoologica, Naples (Italy),
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Attack Elicited by Forebrain and Hypothalamic Stimulation in the Chicken

Defensive reactions, fear and rage have been provoked in mammalian species[1], including man[2], by stimulating brain structures extending from the amygdala to the mesencephalon. Directed attack has been elicited in cats by stimulation of the amygdala[3], the hypothalamus[4–6], the medial thalamus[7] and the mesencephalon[5]. In birds, attack has been elicited by stimulating the hypothalamus and the preoptic area of the pigeon[8] and the brainstem of the chicken[9]. The location of the electrodes is not specified in the aforementioned papers. In ducks attack against

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