notably less active than the butyl derivative[2]. Although not strictly comparable, the 1-β-methoxyethyl derivative (chain length 5.7) has high relative selectivity[3] and in this respect follows roughly the behaviour of the 1-alkyl and 1-aryl derivatives.

This relation between activities and chain lengths suggests that one property of the chemical structure contributing to its activity might increase and another might diminish, with the length of the carbon side-chain at position 1, in such a way that activity could be a maximum at the optimum chain length. Lipid and water solubilities might be 2 such properties and a suitable lipid to water partition coefficient may be of importance.

1-Phenyl-2-(α-hydroxybenzyl)benzimidazole was obtained by fusing an equimolar mixture of *o*-aminodiphenylamine and mandelic acid at 140° for $3^1/_2$ h. The glassy solid so obtained was extracted by hot *M* hydrochloric acid and the extract treated with charcoal and filtered. The cooled filtrate was made alkaline with 3*M* potassium carbonate and the precipitate collected and crystallized from aqueous methanol (with charcoal treatment) to give the *disubstituted benzimidazole* (36% yield) as white prisms, m.p. 184° (Anal.-Found: C, 80.0; H, 5.01; N, 9.5. $C_{20}H_{16}N_2O$ requires C, 80.0; H, 5.33; N, 9.3%). The *hydrochloride* crystallized from 2*M* hydrochloric acid as off-white prisms, m.p. 139.5–141°.

1-β-Methylpropyl-2-(α-hydroxybenzyl)benzimidazole was prepared from *o*-chloronitrobenzene and β-methylpropylamine via *N*-β-methylpropyl-*o*-nitroaniline and the corresponding diamine by the method previously described[1,2]. The *benzimidazole* was obtained, in 35% yield from the *o*-nitroaniline (32.5% overall from *o*-chloronitrobenzene), as white prisms from aqueous methanol after charcoal treatment, m.p. 174° (Anal.-Found: C, 77.0; H, 7.06; N, 10.2. $C_{18}H_{20}N_2O$ requires C, 77.1; H, 7.14; N, 10.0%). The *hydrochloride* crystallized from ethanolic ether as colourless needles, m.p. 196–7°.

Possessing activities of a high order, the compounds are promising as antiviral agents in spite of their relatively low solubilities in water[6].

Zusammenfassung. 1-Phenyl- und 1-β-Methylpropyl-2-(α-oxy-benzyl)-benzimidazol setzen die Vermehrung des Poliovirus der Arten 1, 2 und 3 in der Gewebekulturzelle stark herab. Die Hemmwirkungen und die Beeinflussung der Toxizitätsverhältnisse der 1-Phenyl-Derivate sind alle grösser, als die der früher untersuchten Benzimidazolene.

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The Effect of Ammonium Ion upon Ribonucleic Acid Polymerase and Guanidine Triphosphatase Activity in Isolated Bovine Endometrial Nuclei

Administration of oestrogen to ovariectomized rats results in an enhanced rate of nucleoside triphosphate incorporation into ribonucleic acid (RNA) by nuclei prepared from uterine tissue.

GORSKI[1] noted that the uterine RNA polymerase activity was increased and the difference between oestrogen-treated and control nuclei was lost when concentrations of high ionic strength were present in the medium. Since TATA and WIDNELL[2] proposed the existence of 2 RNA polymerases in thyroid nuclei, one of which was Mg^{++} and the other Mn^{++}/NH_4 dependent, the effect of ammonium ions upon uterine RNA polymerase was examined.

Nuclei isolated from cow endometrium were used. This offers an advantage because in contrast to nuclear preparations from total rat uterus or myometrium of cows, nuclei prepared from bovine endometrium appear microscopically to be devoid of myofibrils.

Cow uteri were supplied by a commercial source. The organs were immediately chilled and were used within 3–4 h after slaughter. The endometrium was collected by scraping. The stage of the ovarian cycle in cows was ascertained by the fern test of the cervical mucous, confirmed by gross and microscopic inspection of the ovaries.

Minced tissues were homogenized with a Lourdes Omni Mix at 5000 rpm in a medium containing 0.32*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 3 m*M* $MgCl_2$. The crude nuclear fraction was separated by centrifugation at 800 *g* and resuspended in 2.4*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 1 m*M* $MgCl_2$. This homogenate was spun at 25,000 *g* for 90 min at − 2 °C, and the pellet was suspended and washed twice in 0.25*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 4 m*M* $MgCl_2$.

RNA polymerase activity was assayed by measuring the conversion of ^{14}C-nucleoside triphosphates into acid-insoluble material[3] under conditions that gave a linear rate of isotope incorporation.

Reactions to determine RNA polymerase activity were carried out in a final volume of 0.5 ml. The components were, unless stated otherwise: 0.2 ml nuclear suspension (100–400 μg DNA), 2.5 μ*M* $MgCl_2$, 0.5 μ*M* $MnCl_2$, 3 μ*N* NaF, 100 μ*M* *Tris* HCl (pH 7.8), 35 μ*M* KCl, 0.3*M* $(NH_4)_2SO_4$, 35 m*M* dithiotreitol, 50 μ*M* uridine triphosphate (UTP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and adenosine triphosphate (ATP), one of which was replaced by either 0.1 μc of ^{14}C-UTP (S.A. 145 mc/m*M*) or 0.1 μc of ^{14}C-GTP (S.A. 25 mc/m*M*) (Schwarz Bioresearch Laboratories. Orangeburg, New York, USA).

The nuclei were pre-incubated for 15 min at 37 °C prior to addition of radioactive isotope in order to exhaust the endogenous pool of nucleotides. Preliminary experiments had shown that the RNA polymerase activity was dependent upon the presence of all 4 nucleoside triphosphates. The reactions were terminated by addition of ice-

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cold 5% tri-chloro-acetic acid (TCA). The precipitates were washed 5 times with 5% TCA and twice with acetone on glass fiber discs with 45 nm pore size. Counting was performed in a Packard tricarb scintillator spectrophotometer with 52.6% efficiency for ^{14}C. Ten ml of Liquifluor (Pilot Chemicals, Watertown, Mass., USA) were used as counting fluid. The results are expressed as cpm/mg DNA, as determined by the method of SCHNEIDER[3]. All values have been corrected for background radiation and zero time incubation.

The time course of polymerase activity of cow endometrial nuclei in the presence of Mg^{++}, Mg^{++} and Mn^{++}, and Mg^{++}, Mn^{++}, NH_4^+, is presented in Figure 1. In the presence of Mg^{++}, the total incorporation of radioactive precursor is low. It increases somewhat when Mn^{++} is present in the medium in addition to Mg^{++}. Although the total incorporation increases severalfold in the presence of NH_4^+, Mg^{++}, and Mn^{++}, there is no significant difference in the initial rate of RNA synthesis.

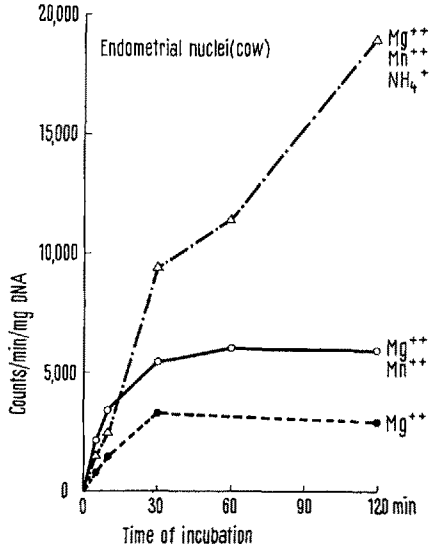

Fig. 1. Kinetics of RNA synthesis of cow endometrial nuclei in the presence of ions, as determined by the incorporation of ^{14}C-UTP into RNA. Incubation was performed at 37 °C. For remainder of experimental conditions, see text.

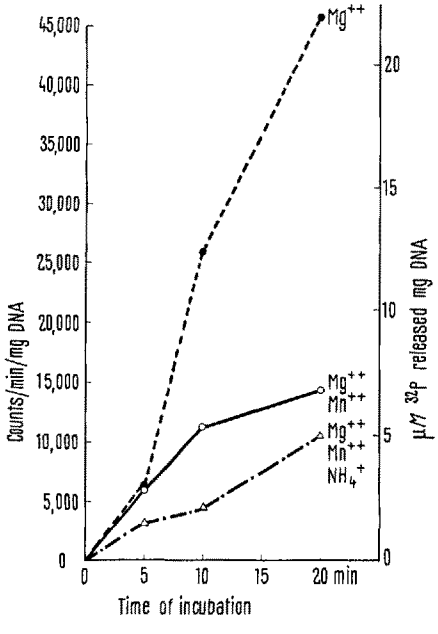

Fig. 2. GTPase activity of cow endometrial nuclei in the presence of ions. ^{32}P-GTP was used as radioactive isotope. The energy regenating system was omitted. For other experimental conditions, refer to text.

The absence of the ammonium ion, the incorporation is linear for a short time, it starts to decrease after 10 min, and plateaus after 30 min. Addition of the ammonium ion, however, results in a linear reaction up to 2 h. These findings suggest that, in the absence of Mn^{++} and NH_4^+, the reaction is limited after a short period of time, but the similarity of initial rates under these 3 conditions indicates that only 1 enzyme is involved.

Since it is known that even purified preparations of RNA polymerases contain nucleoside triphosphatases, the guanosine triphosphatase (GTPase) activity of the nuclear preparations was measured by using 50 µg of GTP containing 100,000 cpm of gamma-labeled ^{32}P-GTP/tube with the other nucleotides in equimolar amounts. The kinetics of the GTPase activity (Figure 2) were determined according to CONWAY and LIPMAN[4].

As can be seen in Figure 2, the GTPase activity is highest in the presence of Mg^{++}. It is less active when Mn^{++} is added to Mg^{++}, and the lowest rate is obtained by the further addition of NH_4^+. The results indicate that NH_4 inhibits the GTPase. After 20 min of incubation, approximately 20% of the available GTP has been hydrolyzed, while during the same period only about 1% of the GTP had been incorporated into RNA. There are obviously at least 2 enzymes contained in these nuclear preparations, namely, RNA polymerase and GTPase, competing for the same substrate, and the latter enzyme having a 20-fold higher rate of activity.

Since large amounts of GDP are formed in the presence of GTPase, the question was raised whether or not GDP is an inhibitor of the RNA polymerase reaction. Substitution of GTP by various amounts of GDP in equimolar proportions did not alter the course of the reaction.

These results are consistent with the hypothesis that the activation of RNA polymerase by Mn^{++} and NH_4^+ is due to the suppression of GTPase by NH_4^+ ion and is not due to the effect of different RNA polymerases[5].

Zusammenfassung. Die Wirkung von 0,3 *M* $(NH_4)_2SO_4$ auf die RNS Polymerasen und GTPasen Aktivität wurde in einem System isolierter endometrialer Zellkerne bovinen Ursprungs untersucht. Bei Zugabe von NH_4^+ verlief die RNS-Polymerasen Reaktion für 2 h linear, anstatt nach 20–30 min in ein Plateau einzumünden. Dies beruht nicht auf einer zweiten RNS-Polymerase, sondern auf der Hemmung der GTPase, welche das Substrat mit wesentlich höherer Reaktionsgeschwindigkeit umsetzt.

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[5] We thank Dr. J. ILAN and Dr. S. J. SEGAL for their advice and help.