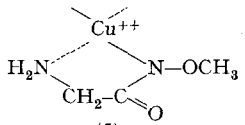
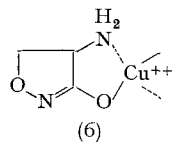
hundredfold[6]. The Table shows the metal-binding constants of GMA and those reported for cycloserine by NEILANDS[4]. Here again there is a very large difference in a biologically important property. GMA is a much stronger metal-binding agent than cycloserine and even binds Cu^{++} more tightly than EDTA (Ks = 18.8)[7]. This difference between cycloserine and GMA may reside in the fact that Cu^{++} can be bound between 2 *nitrogen* atoms (5) in GMA while only a less stable type[8] of binding between the nitrogen and oxygen atoms of cycloserine (6) is sterically possible. The stability order, $Cu^{++} > Co^{++} > Zn^{++}$, of these ions with GMA is consistent with N,N-binding in the complexes[8]. There was, however, very little difference in the visible spectra of the two copper chelates: GMA, λ^{H_2O} = 675 nm (log ε, 1.64); cycloserine, λ^{H_2O} = 700 nm (log ε, 1.45).



(5)



(6)

	Cu^{++}	Zn^{++}	Co^{++}
Cycloserine	9.7	6.0	5.7
GMA	22.2	9.8	16.6

We found also that GMA formed a crystalline Schiff base with 5-chlorosalicylaldehyde under the same mild conditions which gave a cycloserine Schiff base[1a]. We have not investigated the chemistry of this compound further, but its facile formation indicated that GMA could react in vivo with pyridoxal in the same manner that cycloserine most probably does.

In summary, we have found that when the functional groups of cycloserine are arranged in an acyclic structure, no antibiotic activity is observed. This remarkable total loss of activity may be due to a requirement for the ring in the reaction sequence leading to enzyme inhibition or it may be that the large differences in ionization and metal-binding propensities between the cyclic and acyclic compounds lead GMA into biological pathways far removed from the cycloserine site of action[9].

Zusammenfassung. Methoxy-glycinamid, ein offenkettiges Isomeres von Cycloserin, wurde hergestellt. Seine physikalischen Eigenschaften sind von denjenigen des Cycloserins stark verschieden. Es besitzt keine antibiotische Eigenschaft mehr.

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Presence of Sialopolysaccharidic Components in Egg Gelatinous Mantle of *Rana latastei* and *Bufo vulgaris*

CHIARUGI[1] first reported that frog spawn of *Rana esculenta* was able to give metachromasia with aniline basic dyes. RE[2] confirmed the metachromasia in egg gelatinous mantle of *R. esculenta* and *Bufo vulgaris*. A large review on the histochemistry and morphology of Amphibian spawn has recently been published by GHIARA[3]. GIACOSA[4] demonstrated the presence of reducing substances. WOLFENDEN[5] reported the presence of nitrogen-containing reducing substances in egg jelly mucins of *R. temporaria*. SCHULTZ et al.[6] found glucosamine together with other reducing substances. The presence of *d*-galactose[7–9] and fucose[10] has been confirmed by FOLKES et al.[11]. This group was also interested in separating the hexosamines (glucosamine and galactosamine). MINGANTI was interested in the chemical analysis of egg gelatinous mantle from *B. vulgaris, R. esculenta, Discoglossus pictus, Axolotl* and *Triton cristatus*[12–15]; recently MINGANTI[16] reported comparative data on the chemical composition of Amphibian egg mucins.

From the analysis reported on the chemical composition of egg casings we have not found data on the presence of sialic acids, so frequently described as constituents of glycoproteins.

In the present paper we report the data obtained during researches carried out in order to investigate the

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presence of sialic acids in Amphibian egg gelatinous mantle and to confirm the nature of hexosamines.

Experimental preparation and analysis of mucins. The researches were carried out on egg gelatinous mantle of *R. latastei* (collected near Pavia, Lombardy) and *B. vulgaris* (from Caldonazzo Lake, Trentino), treated with UV-rays (10 min) in order to depolymerize the mucins[2] (Ruffini's phenomenon, as reported by BENEDETTI[17] and BRUGI[18]), so that we were able to separate the eggs from the mucins quite easily by filtration through gauze[2]. From the mucins an acetonic powder was prepared. This powder was first analysed for the hexosamines (after 4 *N* HCl hydrolysis, with or without purification through Dowex 50 (H^+) columns, as suggested by a modification[19] of the method of BOAS[20]), for the sialic acid (by the method of SVENNERHOLM[21]) and for the total nitrogen content (following MINGANTI and ZILVERSMITH[22]). Results of preliminary analysis are reported in Table I. We attempted to isolate the polysaccharidic components from proteins by digestion with papain of the acetonic powder suspended in phosphate buffer 0.1 *M*, pH 7.4. The polysaccharidic components were purified following a procedure previously described[23].

On this material we performed the following analysis: determination of hexosamines[19], sialic acids[21], uronic acids[24], hexoses[25], fucose[26], SO_4^{--}[27], and determination of proteins[28]. Chromatographic separation of hexosamines was obtained with the aid of an amino acid analyser Spinco Beckman Model 120 B, on polysaccharidic material after hydrolysis with 4 *N* HCl for 5 h at 105 °C.

Results and conclusion. From the values reported in Table I, where the data of preliminary tests are summarized, the high sialic acid content of the egg mucins is quite evident.

Table I. Preliminary analysis on egg gelatinous mantle

	Rana latastei	*Bufo vulgaris*
Hexosamines	12.9 (16.3)	13.0 (16.9)
Sialic acid	2.2	3.7
Nitrogen	7.4	6.9

The values are given as % of the acetonic powder. The figures enclosed in brackets are calculated from estimations of hexosamines without previous purification on ion exchange resin.

Table II. Analysis of the polysaccharidic fraction isolated from egg gelatinous mantle

	Rana latastei	*Bufo vulgaris*
Hexosamines	16.9	20.9
Sialic acid	7.7	11.1
Hexoses	23.0	30.9
Fucose	8.8	8.1
Uronic acids	ass.	ass.
SO_4^{--}	traces	traces
Proteins	6.8	7.8
Glucosamine/galactosamine ratio	1:1.3	1:8.8

The values are given as % of the polysaccharidic fraction dried over $CaCl_2$.

This is confirmed from the data of Table II, where the analyses are compared of the polysaccharidic components from *R. latastei* and *B. vulgaris* (sialic acid figures are 7.7 and 11.1% respectively). The absence of glucuronic acid and SO_4^{--} is in favour of the absence of sulphated acidic hexosaminoglycuronoglycans. The high fucose content (8%) may suggest a comparison between egg mucins and other glycoproteins described in mammals. In the polysaccharidic fraction isolated from *R. latastei*, glucosamine is slightly more prevalent than galactosamine

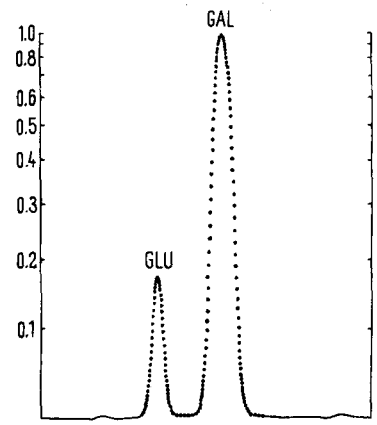

Fig. 1. Hexosamines in the polysaccharidic fraction of egg gelatinous mantle from *Bufo vulgaris*. Glu = Glucosamine; Gal = Galactosamine.

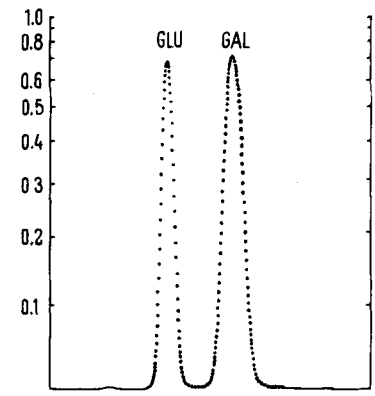

Fig. 2. Hexosamines in the polysaccharidic fraction of egg gelatinous mantle from *Rana latastei*. Glu = Glucosamine; Gal = Galactosamine.

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(1:1.3), as previously suggested by FOLKES et al.[11], meanwhile in the polysaccharidic fraction from *B. vulgaris* there is a much larger amount of galactosamine (glucosamine-galactosamine ratio 1:8.8) (Figures 1 and 2).

It seems to us that in the egg gelatinous mantle of these 2 species sialofucopolysaccharides are present with variable amounts of hexosamines (glucosamine and galactosamine), indicating a different specific composition of the sialopolysaccharides.

Riassunto. Sono stati studiati con analisi biochimiche gli involucri ovulari di *R. latastei* e *B. vulgaris*, soprattutto in relazione alla presenza di esosamine e acidi sialici. Sono presenti sia glucosamina che galattosamina con un rapporto in *R. latastei* di 1:1,3 e in *B. vulgaris* di 1:8,8. Gli acidi sialici sono contenuti in quantità elevata così pure il glucosio, mentre sono assenti acido glucuronico e SO_4^{--}. Si è potuto quindi concludere che negli involucri ovulari delle due specie considerate sono presenti sialofucopolisaccaridi con quantità variabili di esosamine.

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Transfer of the Immunization to a Bacterial Antigen by RNA

By experiments on the transfer of nucleic acids between different cellular populations, data have been obtained on the acquisition of new immunological properties by normal lymphoid cells after treatment with RNA or subcellular fractions extracted from immunized animals[1-5]. It has also been proved that spleen cells from normal mice acquire the ability to synthesize sheep hemolysin after incubation in vitro with RNA extracted from the spleens of isologous donor mice immunized by sheep erythrocytes[6-8]. The acquisition of the immunological capabilities is strictly specific. Thus the ability to synthesize sheep hemolysin is absent in spleen cells from normal mice incubated with RNA obtained from donors immunized with chicken erythrocytes or bovine serum-albumin[8]. The relation between the extracellular-RNA and antibody-globulin synthesis is proved also by the demonstration of an evident increase of the content of RNA in the γ-globulin fraction in immune sera[9].

Immunization transfer in vivo by means of RNA has also been proved feasible by a research programme performed in our laboratory. As a matter of fact, when RNA obtained from the spleens of animals that had been immunized with ram erythrocytes was injected into normal animals, antibodies that could agglutinate ram erythrocytes were found in the latter's serum[10]. Analogous results have been obtained in different experimental conditions. In fact it has been demonstrated that the RNA extracted from the serum of immunized animals is capable of eliciting in normal recipient animals the production of antibodies against the same antigens used for immunizing the animals from which this RNA-immuno-carrier was taken[11].

The research work related in this paper was aimed at assessing the possibility of using RNA for transferring immunization against a bacterial antigen from one animal to another. In this class of investigations, the most serious difficulty arises from the limited amount of RNA that can be obtained from the spleens of immunized animals, particularly when only the nucleolar RNA 2nd fraction is used as the portion endowed with the highest activity. On account of this, our experiments were performed by extracting RNA from the spleens of a large number of immunized adult subjects and injecting it into new-born animals so as to obtain the highest possible RNA concentration in the plasma of the recipient subjects. Animals originating from the same stock were used throughout the experiment.

Methods of immunization and RNA extraction. 50 rabbits averaging 3 kg in weight were administered 5 i.v. injections (1 every third day), each consisting of 5 ml of a suspension of *S. typhi* 'H' antigen. The antigen had been prepared from agar cultures of *S. typhi* H 901 treated with formalin, the opacity being equivalent to that of the first tube of Wellcome's opacity meter. 20 days after the beginning of the immunizing treatment, antibody titrations were in excess of 1/70,000 in all subjects. At that time the rabbits were killed and RNA was immediately extracted from their spleens by the GEORGIEV and MANTIEVA method[12,13].

Characteristics of the RNA employed. In the experiment only nucleolar RNA 2nd fraction was used. The chemical composition of this fraction was as follows: RNA 96.8%; proteins 1.5%; polysaccharides 0.8%; DNA 0.9%. For the control of the molecular integrity of RNA it was characterized by the hyperchromic effect: Optical density (OD) of the native RNA 2.07 (260/280 nm); 2.26 (260/230 nm); OD after alkaline hydrolysis 1.81 (260/280 nm); 2.41 (260/230 nm).

Treatment of the recipient rabbits. Some of the RNA so obtained was dissolved in a salt-free 5% dextrose solution and injected i.v. (dosage: 10 mg/kg body weight) into 5 new-born rabbits. The injections were repeated

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