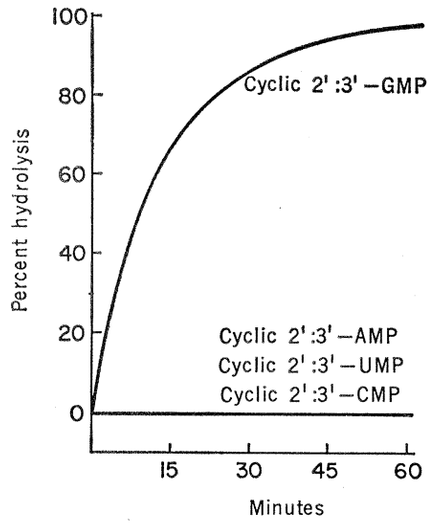

Fig. 2. Hydrolysis of cyclic 2′:3′-nucleotides by ribonuclease Ch. Cyclic 2′:3′-AMP, UMP, CMP, and GMP were obtained from Schwarz BioResearch as their barium salts. Barium was removed with an equivalent quantity of sodium sulfate, and cyclic nucleotides were diluted to 0.002*M*. One-milliliter samples were titrated in a Radiometer Titrigraph TTT-1, and 0.005*N* NaOH was used to maintain a *p*H of 7.0. The reactions were started by adding 50 μg of ribonuclease Ch protein. After alkali consumption had ceased, the samples were chromatographed in isopropanol, saturated ammonium sulfate, water (2 : 79 : 19, by volume) (*12*). In the case of cyclic 2′:3′-GMP, there was quantitative conversion to 3′-GMP. The other cyclic 2′:3′-nucleotides were unchanged.

poses. Eastman thin layer chromatograms, and silica gel without fluorescent indicator (K 301R2), were activated 30 minutes at 110°C, and aliquots of the enzymatic digest were streaked along a line 1 cm from the bottom. Separation was accomplished with the first-dimension solvent named in the legend to Fig. 1. The fastest-moving band, which corresponded to authentic guanosine, was eluted with 0.25*M* ammonium formate, *p*H 4.1. Its ultraviolet absorption spectrum was identical to that of authentic guanosine.

Results in Fig. 1 and those from column chromatography indicate an absolute specificity of ribonuclease Ch for 3′-GMP residues. In both cases the results for the longest incubation period were identical with those obtained with an incubation period of 10 minutes. Thus, with an amount of ribonuclease Ch sufficient to give maximum hydrolysis in 10 minutes, no further hydrolysis of nucleotide bonds occurred in 16 hours.

Ribonucleases from other sources hydrolyze RNA through the intermediate cyclic 2′:3′-nucleotides (*7*). Ribonuclease Ch presumably acts in the same manner because cyclic 2′:3′-GMP is hydrolyzed to 3′-GMP (Fig. 2). These data also indicate an absolute specificity for guanosine, because the other cyclic 2′:3′-nucleotides were unaffected by ribonuclease Ch.

Evidence was sought for the presumed intermediate, cyclic 2′:3′-GMP. For this purpose, polyguanylic acid, (Miles Laboratory) was used as substrate, and the reaction was monitored by alkali consumption in a Radiometer Titrigraph TTT-1. At intervals, aliquots were removed and chromatographed in the system in the legend to Fig. 2. In the first 5 to 15 minutes of hydrolysis there were small spots corresponding to cyclic 2′:3′-GMP, but in longer periods of time the only spots observed were those corresponding to 3′-GMP. Cyclic 2′:3′-GMP does not accumulate, which indicates that the rate-limiting reactions are those concerned with the hydrolysis of the 3′-5′ linkages in polyguanylic acid.

Ribonuclease Ch thus has the same specificity as ribonuclease T_1 (*8*), N_1 (*9*), and U_1 (*10*). Ribonuclease T_1 has proven to be of great value in structural studies of nucleic acids, and ribonuclease Ch may also have similar applications. It will also be of interest from the standpoint of comparative enzyme structure.

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Serum Copper Alteration after Ingestion of an Oral Contraceptive

Abstract. *Changes in the concentration of copper in the serum after administration of an oral contraceptive were determined with atomic absorption spectrophotometry. Statistically significant* ($P = .001$) *increases were observed in all volunteers.*

Concentrations of heavy metals and trace elements in serums of women taking the oral contraceptives have not been well studied. We now report the use of the atomic absorption spectrophotometer to determine changes in total serum copper before and 1 month after administration of an oral contraceptive.

Fourteen healthy female subjects, more than 6 weeks after parturition were tested before ingestion of 10 mg of norethynodrel with mestranol daily for 21 days; hence they served as their own controls. Venous blood was again obtained after this cycle. All specimens were obtained from fasting individuals and were run in duplicate.

Prior to treatment, the mean concentration of copper in the serum was 142 μg per 100 ml with a range of 104 to 168 μg per 100 ml, and the standard error of the mean was 12.0 μg per 100 ml, a range consistent with value in the serums of women of childbearing age. After one cycle of administration of oral contraceptive the mean rose to 241 μg per 100 ml with a standard of the mean of 7.3 and a range of 184 to 296 μg per 100 ml. The *t* value was 6.607, and *P* was .001.

These observations demonstrate a marked increase in the concentrations of serum copper after ingestion of an oral contraceptive for only 21 days. The change is statistically significant. The long-term effects of this alteration in serum copper content remain to be determined. The mechanism of action of this increase is unknown but may represent a variation in the plasma proteins that bind the various metals. These findings may be significant for two reasons. (i) They may help to explain the changes demonstrated in normal pregnancy, and (ii) they may point to a potential long-term hazard.

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