denitrificans which contain a major proportion of unsaturated fatty acids (Table II). One of the striking features of the fatty acid complement of *M. radiophilus* is the large quantity of even numbered poly-unsaturated acids. The occurrence of polyunsaturated fatty acids in bacteria is rare—these poly-unsaturated acids have so far been reported mainly in *Bacillus* sp.[5]

The outstanding differences in the fatty acid spectrum of *M. radiophilus*, as compared with those of other micrococci, support our earlier suggestions[12, 14], that the placement of this bacterium in the genus *Micrococcus* should be reconsidered.

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1. Abel, K., DeSchmertzing, H. and Peterson, J. I., *J. Bacteriol.*, 1963, **85**, 1039.
2. Anderson, A. W., Nordan, H. C., Cain, R. F., Parrish, G. and Duggan, D., *Food Technology*, 1956, **10**, 575.
3. Cho, K. Y. and Salton, M. R. J., *Biochim. Biophys. Acta*, 1966, **116**, 73.
4. Fulco, A. J., Levy, R. and Bloch, K., *J. Biol. Chem.*, 1963, **239**, 998.
5. —, *Ibid.*, 1969, **244**, 889.
6. Girard, A. E., *Can. J. Microbiol.*, 1971, **17**, 1503.
7. Hancock, I. C. and Meadow, P. M., *Biochim. Biophys. Acta*, 1969, **187**, 366.
8. Kates, M., *Adv. Lipid Res.*, 1964, **2**, 17.
9. Knivett, V. A., Cullen, J. and Jackson, M. J., *Biochem. J.*, 1965, **96**, 2c.
10. Kobatake, M., Tanabe, S. and Hasegawa, S., *C.R. Soc. Biol. (Paris)*, 1973, **167**, 1506.
11. Lewis, N. F., *J. Gen. Microbiol.*, 1971, **66**, 29.
12. Lewis, N. F., *Curr. Sci.*, 1973, **42**, 504.
13. Matsuyama, A., In *Radiation Preservation of Food*, IAEA, Vienna. 1973, pp. 261,
14. Sleytr, U. B., Silva, M. T., Kocur, M. and Lewis, N. F., *Arch. Microbiol.*, 1976, **107**, 313.
15. Yano, I., Furukawa, Y. and Kusunose, M., *Biochim. Biophys. Acta*, 1969, **187**, 166.

DIFFERENTIAL PRECIPITATION OF GLYCOSAMINOGLYCANS BY ZINC AT pH 7·0

WHILE studying zinc binding by each of the seven different glycosaminoglycans (GAGs)[1], we observed that when slightly acidic solutions containing the sodium salt of a GAG and $^{65}ZnCl_2$ were adjusted to pH 7·0, the amounts of ^{65}Zn precipitated were relatively constant, leaving 41·4 to 49·0% of the total ^{65}Zn in the supernates (Table I). However, in contrast to ^{65}Zn, the amounts of GAGs remaining in the supernates ranged from 3·8 to 90·2% of the total GAGs.

TABLE I
Percentages of GAGs and ^{65}Zn found in pH 7·0 supernates

Samples	GAGs (%)	^{65}Zn (%)
Chondroitin-4-sulfate	68·1	43·4
Chondroitin-6-sulfate	48·5	42·8
Dermatan sulfate	64·3	42·9
Heparan sulfate	3·8	41·4
Heparin	7·8	47·0
Hyaluronic acid	73·1	49·0
Keratan sulfate	90·2	45·5

The values reported in Table I were obtained from samples containing 600 μg of a specified, reference standard GAG (gift of Dr. J. A. Cifonelli, University of Chicago) and 10 μmoles of $^{65}ZnCl_2$. Each sample was adjusted to pH 7·0 by the addition of dilute NaOH and made to 320 μl with water. After setting aside at room temperature for 15 min and centrifuging, the supernates were measured for hexuronic acid content by the *m*-hydroxydiphenyl method[2] and galactose by the anthrone method[3]. The radioactivity of ^{65}Zn was measured with the Packard Gamma Scintillation Spectrometer. Aliquots of a working solution of $^{65}ZnCl_2$ were used as reference standard. The working solution of $^{65}ZnCl_2$ was prepared by adding an aliquot of $^{65}ZnCl_2$ (1–10 Ci/g zinc in 0·5 N HCl, purchased from New England Nuclear, Boston, Massachusetts 02118) to an aqueous solution of $ZnCl_2$, adjusting to pH 4·0 with 0·01 N NaOH and diluting to 1·000 M zinc chloride, inclusive of $^{65}ZnCl_2$. Each GAG was dissolved in glass-distilled deionized water, adjusted to pH 4·0 with NaOH, and diluted to a concentration of 2·0 mg GAG/ml.

Appropriate precautions were taken with glassware and equipment to prevent contamination with extraneous zinc. Syringes (Hamilton Co., Reno, Nevada) were washed with 0·02 N HCl and rinsed thoroughly with water and with the solution to be measured just before use.

Because zinc is an ampholyte (having both acidic and basic functions in presence of strong bases and strong acids respectively[4]), its solubility in acidic and basic solutions was ascertained. At pH 4·0, 10 μ

moles of $^{65}ZnCl_2$ produced no precipitate in 1·0 ml of 0·1 M NaCl solution. At pH 7·0, 4·0 μ moles of ^{65}Zn were found in the supernate and this value was not affected by NaCl concentrations of 5×10^{-6} to 10^{-1} M. Minimum solubility of ^{65}Zn in 0·1 M NaCl was observed at pH 11·0; less than 0·1 μmole of ^{65}Zn/ml was found in the supernate. In the absence of zinc, but otherwise identical conditions, GAGs did not form any precipitate.

The differential precipitation of GAGs was observed at pH 7·0, where 51·0 to 58·6% of the total zinc had sedimented. Thus, one might expect that there was some coprecipitation of GAG with zinc. However, the wide range in GAG solubility (3·8 to 90·2%) indicates that coprecipitation was not purely mechanical. Rather, it appears that GAG solubility in neutral zinc solution is roughly related to GAG composition and structure[5] (Table I): Keratan sulfate, the least precipitable GAG, has practically no hexuronic acid (1·9%) by weight, given with the sample), unlike the other GAGs. The most precipitable GAGs, heparan sulfate and heparin, are the only GAGs having sulf-amino groups; they also have ester sulfate groups and hexuronic acids. Hyaluronic acid has hexuronic acids but no ester sulfates; only 27% of the total was precipitated. Chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate have hexuronic acids and ester sulfates; intermediate amounts of these GAGs were precipitated. The GAG solubility, in addition, seems partly related to zinc-binding capacity[1]: Of the GAGs, keratan sulfate binds lowest amounts of zinc; heparin binds highest amounts (heparan sulfate, slightly less) of zinc; and chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate bind intermediate amounts of zinc. Hyaluronic acid also binds highest amounts of zinc, but unlike heparin, its zinc-binding capacity decreases greatly with decreasing pH. Zinc-binding capacity of dermatan sulfate, while less than that of chondroitin-6-sulfate, approximates that of chondroitin-4-sulfate. (This trend also appeared in Table I.)

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1. Sato, C. S. and Gyorkey, F., *J. Biochem.*, 1976, 80, 111.
2. Blumenkrantz, N. and Asboe-Hansen, G., *Anal. Biochem.*, 1973, 54, 484.
3. Spiro, R. G., *Meth. Enzymol.*, 1966, 8, 3.
4. Partington, J. R., *A Text-Book of Inorganic Chemistry*, 6th Edition, MacMillan, London, 1950, p. 780.
5. Balazs, E. A., *Chemistry and Molecular Biology of the Intercellular Matrix*, Vol. 2, Academic Press, New York, 1970, p. 705.

EFFECT OF CHLORMADINONE ACETATE ON THE WEIGHT AND CHOLESTEROL CONTENT OF THE RAT ADRENAL

USE of the potent synthetic progestin chlormadinone acetate (6-chloro-6-, 17α-acetoxyprogesterone) has become widespread in clinical contraception[6]. Recently chlormadinone acetate has also been shown to produce functional sterility in the male rat[2,7]. While examining the antifertility effects of low doses of chlormadinone acetate in the male rat it was considered worthwhile to investigate if the progestin had any effect on the adrenal.

Wister albino male rats (300–325 g body wt.) of proven fertility from the departmental colony were used for this study. The control and 3 experimental groups each contained 6 animals which were housed individually. Animals in the experimental groups received a daily intramuscular injection of 0·2, 0·5 or 1·0 mg chlormadinone acetate in 0·1 ml olive oil for 40 days. Controls were similarly injected with the vehicle alone. Autopsies were made 24 hr after the last injection. Adrenals were taken out, cleared from adhering tissue and weighed to the nearest mg. The total cholesterol content of the adrenals was quantitatively determined by the method of Zak as described by Hawk *et al*[6]. The results were analysed by "Student's" *t*-test[3].

With increasing doses of chlormadinone acetate, the weight of the rat adrenals correspondingly decline (Table I). However, the total cholesterol content of the adrenals increases with the dose and a statistically highly significant increase ($p < 0·001$) results at the 1·0 mg/day dose.

There is substantial experimental evidence to support the view that the most important precursor of steroidal hormones, whether adrenal[1] or gonadal[4], is cholesterol. Effect of chlormadinone acetate on the adrenals of the rat could be visualised in this light. Thus the progestin may possibly influence synthesis of adrenocorticoids from cholesterol by blocking the enzyme system at one or more steps of the biosynthetic pathway causing accumulation of cholesterol and depletion of steroids, the latter being one of the important possible causes of the decrease in weight of the gland. Due to lack of experimental evidence it is difficult