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

TABLE I

Effect of chlormadinone acetate for 40 *days on the weight and cholesterol content of the adrenal of the male rat* 6/group, mean value ± S.E.M.

Group	Total weight (mg)	Total cholesterol (mg/gm tissue)
Vehicle control	14·15±1·09	15·3±1·59
Chlormadinone acetate:		
0·2 mg/day/rat	13·52±1·23	19·3±2·13
0·5 mg/day/rat	10·63±0·45*	31·6±2·57**
1·0 mg/day/rat	10·47±1·01*	37·9±3·27***

Significantly different compared with control: * $p < 0·05$; ** $p < 0·01$; *** $p < 0·001$.

to surmise whether the inhibitory action of chlormadinone acetate on the rat adrenal is direct or indirect. It may be mentioned that increasing doses of chlormadinone acetate which were employed in this experiment also caused a complimentary dose-dependent decrease in the weight and gonadotropin content of the pituitary along with gonadal function[7]. A similar type of mediation of action of chlormadinone acetate through the hypophyseal-adrenal axis can be conjectured although on a purely hypothetical basis.

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DENNIS JACOB.

1. Dorfman, R. I., *Cancer*, 1957, 10, 741.
2. Dörner, G., Gotz, F. and Mainz, K., *J. Endocr.*, 1972, 52, 197.
3. Fisher, R. A., *Statistical Methods for Research Workers*, Hafner Publ. Co., N.Y., 1958.
4. Hall, P. F., *The Testis*, Vol. II, Academic Press, N.Y., 1970.
5. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry*, McGraw-Hill, N.Y., 1954.
6. Martinez-Manautou, J., Giner-Velasquez, J. and Rudel, H., *Fertil. Steril.*, 1967, 18, 57.
7. Sharma, M. M., Lal, G and Jacob, D., *J. Reprod. Fert.*, 1976, 48, 177.

EXPOSURE OF WHITE MICE TO *BACULOVIRUS AMSACTA* OF GROUNDNUT RED-HAIRY CATERPILLAR, *AMSACTA ALBISTRIGA* (WALKER)

RECENT interest in the use of the nuclear polyhedrosis virus (NPV) against the groundnut red-hairy caterpillar, *Amsacta albistriga*[1,2] as a possible microbial agent, necessitates to establish whether this virus is pathogenic and/or toxicogenic to other non-target animals. More evidence is being accumulated on the specificity of insect polyhedrosis viruses and their safety to vertebrates[3]. In India, though the usefulness of NPV of *A. albistriga* has been reported by Jayaraj *et al.*[1,2] there has been no study on its safety against the other non-target animals except the recent study of Narayanan *et al.*[4,5] in which the authors have established the innocuous nature of this virus to common carp, *Cyprinus carpio* and to poultry birds. The present report is the result of a similar study of the possible toxicity and/or pathogenicity of NPV of *A. albistriga* to white mice.

The polyhedral inclusion bodies were processed from dead, final instar larvae of *A. albistriga* by differential centrifugation and the final concentration was characterised as to the number of PIBs/ml. To find out the bacterial contamination of the virus preparation, differential bacterial-plate counts were made. The total number of bacterial cells present was estimated by pour plate technique using Difco Nutrient Agar and the presence or absence of coliforms was detected using Mac-Conkey's agar medium. The number of viable spores present was estimated by pasteurization technique. The acute toxicity/pathogenicity evaluation tests were carried out by following standard methods[6].

Two to four weeks old and randomly mated non-inbred lines of white mice (average weight 26·1 g; range 21·4 to 28·6 g) were used in the study. An oral administration of 0·01 ml of virus suspension was given using a 1 ml tuberculin syringe fitted with 5 cm tifflon sleeve of 0·5 mm dia to each of five male and five female mice. The virus suspension was found to contain 1·9 to 2·5 × 10^8 bacterial cells, 2·1 × 10^3 bacterial spores and no coliforms. The dose fed to each mouse was more than 100 times the average field dose/acre with a conversion ratio of the weight of test animal to weight of man. In addition, ten mice, five in each sex, were used as untreated control, receiving 0·01 ml of sterile distilled water. The animals were maintained in separate cages and standard commercial food material along with sliced carrots and water