

Endrin Induced Alterations in Bound Carbohydrates in Rat Serum

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Introduction

The varied effects of endrin, a highly toxic cyclodiene pesticide, have been recently reviewed (1). In general, endrin manifests its effects through action on the central nervous system. Evidence of additional alterations in the composition of blood serum has been shown by shifts in certain trace metal concentrations following acute (2) and sub-acute (3) oral injection of endrin. The purpose of the present study was to determine if endrin induces alterations in rat blood serum lipoproteins, electrophoretic distribution of serum proteins, and bound carbohydrates. Any alterations in bound carbohydrates would indicate metabolic alterations in serum glycoproteins which as a class could be involved directly or indirectly in the defense and repair metabolism of the blood systems.

Procedure

The experimental animals consisted of adult male Holtzman albino rats having an average weight of 197 g. The animals were randomized into seven groups of ten rats each and allowed to receive water and Rockland Mouse Breeder diet ad libitum. The control animals received peanut oil without endrin and with one group being sacrificed at each of the following time periods: 0, 5, 12 and 19 days. The exposed

groups were sacrificed after having received endrin for 5, 12 and 19 days, respectively. Endrin was prepared in commercial grade peanut oil, 1 mg per ml, and injected intraperitoneally once each day at a dose level of 1 mg of endrin per kg of body weight.

The animals were sacrificed by first placing each under anesthesia with diethyl ether, then opening the abdominal cavity and exsanguinating the animal by inserting a needle attached to a heparinized syringe into the abdominal aorta just above the iliac arch. The blood plasma was separated by centrifugation at 15,000 x g for 5 min.

Protein concentrations were determined by the Folin-phenol method of Lowry et al. (4). Protein bound neutral sugar was analysed by the tryptophan method (5) with mannose and galactose as standards and Winzler's method (6) was employed in the analysis of protein bound hexosamine content. Free and bound sialic acid, as N-acetylneuraminic acid, was determined before and after hydrolysis for 1 hour at 80° C in 0.1 N sulphuric acid according to the method of Warren (7). Free sialic acid was not detected in the serum. Bound methylpentose, as fucose, analysis was performed by the method of Gibbons (8). Electrophoresis on paper was conducted in 0.075 M barbital buffer, pH 8.6, for 16 hours at 5 ma. Duplicate strips were stained for general protein and lipoprotein by the method of Shetlar et al. (9).

Results and Discussion

The paper electrophoresis results for lipoprotein, albumin, α_1 , α_2 , beta and gamma globulins demonstrated no statistical significant differences at the 0.05 level between control and endrin treated

groups. General serum protein concentrations in g per 100 ml were found to be 5.61 ± 0.3 for the control groups as well as the 5-day and 12 day endrin treatment groups and 5.89 ± 0.2 for the 19 day endrin treatment group. This increase in serum general protein concentration was not statistically significant.

Table 1 summarizes the results for the protein bound carbohydrates in the blood serum of the control and endrin treated groups. The four control groups were employed to test the effect of time on the reported values. This set of data was statistically treated, and as no age effect was found the data were treated as one group. The control values in mg per 100 ml with their respective standard deviations were as follows: sialic acid, 86.88 ± 7.14 ; hexose, 149.55 ± 10.98 ; methyl pentose, 12.68 ± 1.23 ; and hexosamine, 172.66 ± 8.63 . As is apparent, no consistent pattern of directional alterations occurred.

TABLE 1

SERUM PROTEIN BOUND CARBOHYDRATES FOR CONTROL AND ENDRIN TREATED RATS

| | <u>Control</u> | <u>5-Day</u> | <u>12-Day</u> | <u>19-Day</u> |
|----------------|----------------|--------------|---------------|---------------|
| Sialic Acid | 86.88 | 100.05* | 101.23* | 90.84 |
| Hexose | 149.55 | 150.04 | 150.76 | 162.03* |
| Methyl Pentose | 12.68 | 13.47 | 15.55* | 14.11* |
| Hexosamine | 172.66 | 156.96* | 155.12* | 157.97* |

* Indicates statistical significance at the 0.05 level. All values are expressed in mg per 100 ml of blood serum and are based on results from 10 animals except the control values which were based on 40 animals. Endrin treated groups received, intraperitoneally, 1 mg endrin per kg body weight per day for the indicated time periods.

Bound sialic acid demonstrated a significant increase by the end of 5 days and remained elevated at the end of 12 days. By the 19th day of continuous daily insult of endrin, bound sialic acid content was approaching control values. Bound methyl pentose values exhibited somewhat the same pattern in that a statistically insignificant increase had occurred by the 5th day and was further increased to a statistically significant value by the 12th day and on the 19th day was showing a tendency toward recovery although it was still significantly elevated. Bound hexose content showed the opposite effect, that is, no change from control values occurred during the initial 12 days, but by the 19th day a significant increase had appeared. Bound hexosamine was significantly depressed by the end of 5 days and remained depressed at the end of each of the other two time periods tested.

All animals had a mean weight of 197 g at the beginning of the experiment. The control animals gained an average of 30, 62 and 89 g by the end of 5, 12 and 19 days respectively, while the endrin treated rats gained an average of 34, 46 and 40 g by the end of 5, 12 and 19 days respectively. Initially, the experimental design was to continue the endrin treatment beyond 19 days, but by the end of 19 days of daily endrin injection, this group of rats had not only failed to gain weight between 12 and 19 days but was beginning to lose weight and had such a poor appearance that the experiment was terminated prior to the obviously approaching forced termination. In view of the state of endrin intoxication of these animals, it was surprising to find no alterations in the various electrophoretic serum protein fractions or lipoproteins, and more dramatic alterations in the glycoproteins might

have been expected.

Summary

Endrin (1 mg per kg body weight) was daily administered intraperitoneally to rats which were sacrificed in groups of 10 after 5, 12 and 19 days and analyses of blood serums were compared to appropriate controls. No alterations were found in the concentrations of general serum protein or paper electrophoretically separated serum lipoproteins, albumin, α_1 , α_2 , beta or gamma globulins. Protein bound sialic acid and methyl pentose had increased by the 5th day, remained elevated through the 12th day and appeared to be decreasing back toward control values by the 19th day although these values were still above normal. Bound hexose demonstrated no changes for the first 12 days but had increased significantly by 19 days. Bound hexosamine was significantly decreased by the end of 5 days and remained at this depressed level for the 19 days.

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