retention time as acetaminophen in GC. The hydrolytic product was identified as acetaminophen by GC–MS (Figs. 1 and 2).

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# Toxicological Approaches to Streptothricin Antibiotics. IV.[1] Toxicity of Streptothricin Antibiotics to the Blood

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The hematoxicity of a streptothricin antibiotic was investigated in rats by blood morphological examination, by scanning electron microscopic observation of changes in the erythrocytic membrane and by means of the coil planet centrifuge (CPC) technique to detect alterations in erythrocytic membrane dysfunction. The administration of the antibiotic caused no appreciable hematological change, nor any alteration in the morphology or function of the red blood cell membrane. The results indicate that the streptothricin antibiotic has no hematotoxic potential.

**Keywords**——racemomycin-D; coil planet centrifuge; erythrocyte; erythrocyte membrane; hemolysis; delayed toxicity; scanning electron microscopic observation

In previous reports from this laboratory, the cause of toxicity of streptothricin antibiotics in mice and rats was investigated by assessments of antibiotic distribution in various organs and tissues,[3] histopathological studies[4,5] and serum biochemical examinations,[3] and a marked nephrotoxic potential of this group of compounds was demonstrated. The animals dosed with the antibiotics showed no significant adverse histopathological changes in the spleen or liver, though the organs showed a marked progressive decrease in weight. These two organs, as well as the kidneys, which exhibited conspicuous pathologic changes, are closely related to the blood. This report describes a study of the toxicological effect of a streptothricin anti-

biotic by hematologic examination, scanning electron microscopic observation of the erythrocytic membrane and the coil planet centrifuge (CPC) testing of the red cell membrane function of blood from rats given the antibiotic.

## Materials and Methods

**Animals**——Male rats of the Wistar strain ranging in weight from 200 to 250 g were used.

**Antibiotic**——Racemomycin-D[6] is a streptothricin antibiotic produced by *Streptomyces lavendulae* OP-2.[7]

**Administration and Dose**——The antibiotic was administered at a dose of 40 mg/kg *via* the tail vein as described previously.[5]

**Collection of Blood Samples**——Samples of blood were drawn from the heart under ether anesthesia at various times after injection of racemomycin-D.

**Hematological Examination**——The erythrocyte and total leukocyte counts, hemoglobin content and hematocrit were determined with a Coulter counter, model S. The platelet count was determined with a thrombocytometer (model PR-100, Tokiwa Co., Ltd.). The copper sulfate method was employed for determination of blood specific gravity.

**Preparation of Specimens for Scanning Electron Microscopy**——Approximately 0.2 ml of whole blood was fixed by dropping it in 1% glutaraldehyde (diluted with Millonig solution). After 30 minutes of fixation, the fixative was removed by centrifugation at 1500 rpm for 5 minutes and the blood was washed six times with Millonig solution by centrifugation at 1500 rpm for 5 minutes, followed by dehydration in a graded series of ethanol (50, 60, 70, 80, 90, 95 and 100%) for 5 minutes at each concentration, with centrifugation at 1500 rpm for 5 minutes. To the dehydrated blood cell specimen in a centrifuge tube, 100% isoamyl acetate was added. The tube was shaken vigorously to disperse the red cells, and one or two drops of the red cell suspension were dropped onto an aluminum foil and immediately placed in a critical point desiccator for drying. The specimen was then subjected to vaccuum evaporation with gold in an ion-coater and examined in a scanning electron microscope (JEM-35C) at 15 Kv, with a magnification of $\times 3000$.

**Measurement of Erythrocytic Fragility (CPC Method)**——A 10 $\mu$l portion of heparinized whole blood sample was slowly injected into a coiled tube (0.3 mm$\phi \times 3$ m) containing NaCl solutions with a density gradient from 30 to 150 mOsm, and the tube was preincubated at 37° for 10 minutes then spun on a CPC centrifuge, model ST. The hemolytic patterns of samples were recorded with an SSP-V scanning spectrophotometer to calculate the starting, maximum and end points of hemolysis, expressed in mOsm.

## Results

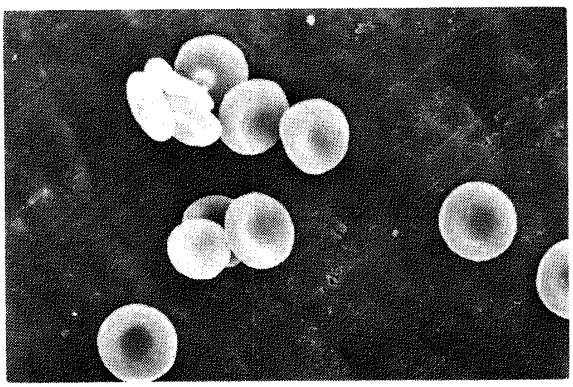
### Hematologic Findings in Rats Following Administration of Racemomycin-D

Serial blood samples obtained from rats following administration of racemomycin-D were examined to determine the hematological parameters (Table I). Though strong nephrotoxicity was found at a dose of 40 mg/kg, as described previously,[5] none of the parameters showed any appreciable change at this dose. No significant change was noted even at 72 hours after injection, by which time pronounced, delayed toxicological effects had developed with occasional deaths.

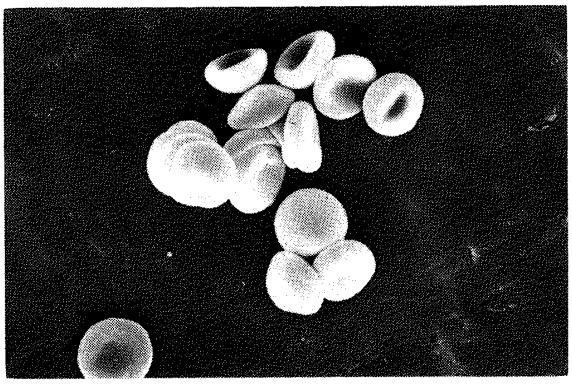
Table I. Hematological Parameters after Racemomycin-D Administration to Rat

| | Control | 5 | 24 | 48 | 72 (hr) |
|---|---|---|---|---|---|
| WBC ($10^3$/mm$^3$) | 9.72±0.4550 | 12.44±0.9723 | 13.45±0.7772 | 11.33±0.4447 | 12.53±0.7060 |
| RBC ($10^3$/mm$^3$) | 7.49±0.0723 | 6.94±0.8870 | 6.77±0.0606 | 7.43±0.0748 | 7.60±0.1029 |
| Hb (g/dl) | 15.08±0.1625 | 13.63±0.2028 | 14.24±0.2050 | 14.41±0.1370 | 14.88±0.1638 |
| Ht (%) | 42.30±0.4899 | 39.40±0.9218 | 41.50±0.4182 | 42.53±0.5993 | 42.58±0.4268 |
| MCV ($\mu^3$) | 59.60±0.8718 | 58.90±0.7951 | 58.70±0.4995 | 58.50±0.2687 | 57.40±0.4269 |
| MCH ($\mu\mu$g) | 19.97±0.1202 | 19.74±0.1275 | 20.28±0.1020 | 20.30±0.1282 | 20.21±0.4410 |
| MCHC (%) | 34.69±0.4368 | 34.09±0.5043 | 33.86±0.1185 | 34.48±0.0917 | 35.05±0.0847 |
| GB | 1057.80±0.4422 | 1054.20±0.4422 | 1054.10±0.6904 | 1056.90±0.3480 | 1060.00±0.8819 |
| BP ($10^4$/mm$^3$) | 92.20±2.3842 | 83.60±4.5976 | 72.20±3.6417 | 104.92±2.1097 | 112.12±2.7907 |

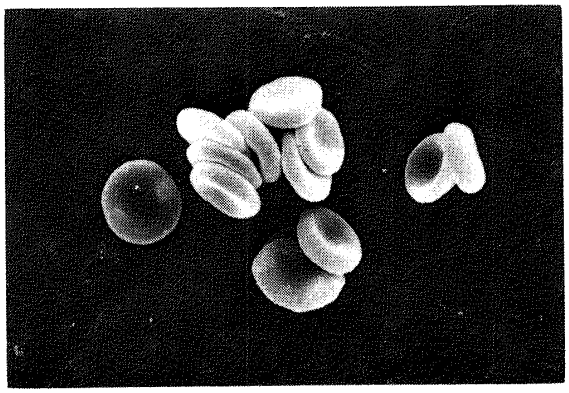
Each value represents the mean ± S.E. of 10 rats.
Racemomycin-D, 40 mg/kg.



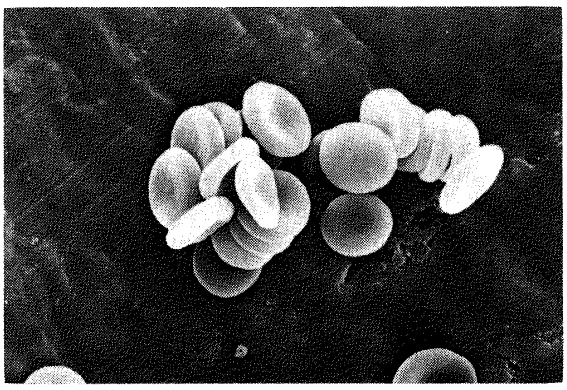
a) control



b) 24 hr after injection



c) 48 hr after injection



d) 72 hr after injection

Fig. 1a—1d. Scanning Electron Micrographs of Rat Erythrocytes after Administration of Racemomycin-D (×3000)

Dose: 40 mg/kg, route: intravenous injection.

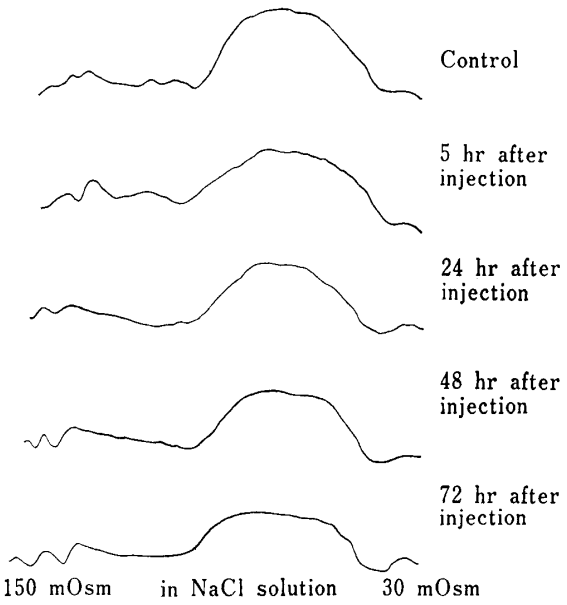



Fig. 2. Hemolytic Pattern as followed by CPC during Intratubular Coagulation

Sample: Blood of rats after administration of racemomycin-D.
Animal: Wistar strain rats (male) 200—250 g (body weight)
Route : Intravenous injection.
Dose : 40 mg/kg.

## Scanning Electron Microscopic Observation of Erythrocytes from Rats after Administration of Racemomycin-D

Scanning electron microscopic observation of erythrocytes obtained from rats at various times after an *i.v.* injection of racemomycin-D was carried out to detect changes in the erythrocytic membrane. The results are shown in Fig. 1a to 1d. Little or no change in the treated group was seen at any period after injection, compared with the control group. The findings in the treated group were comparable with those in the controls even at 72 hours, by which time some of the dosed rats had succumbed.

## Erythrocytic Resistance in Rats after Administration of Racemomycin-D

The resistance of red cells of rats was assessed by the CPC method to investigate the condition of the red cell membrane after racemomycin-D administration. Fig. 2 shows typical hemolytic patterns of blood

Table II. Values of Resistance of Erythrocytes (Osmotic Fragility)

| Time (hr) after administration | Osmotic fragility (mOsm)[a] | | |
|---|---|---|---|
| | Start point | Maximum point | End point |
| Control | 110 | 90 | 60 |
| 5 | 110 | 86 | 62 |
| 24 | 112 | 84 | 61 |
| 48 | 106 | 82 | 61 |
| 72 | 110 | 85 | 62 |

a) Measured at 37°, values are means of 6 samples.
Sample: blood of rats after administration of racemomycin-D.
Animal: Wistar strain rats (male), 200—250 g (body weight).
Route : intravenous injection.

drawn from a control and from a treated rat at various times after injection. In Table II, the data obtained for osmotic fragility of erythrocytes are presented.

As can be seen from Fig. 2, exactly the same hemolytic pattern as in the control was noted in the treated rats at all periods. There was no difference at all in the osmotic fragility of red cells between the control and treated groups at any time (Table II), nor was there any marked change in the treated group even at 72 hours after injection, by which time the animals had developed manifestations of marked delayed toxicity and some had died.

## Discussion

Generally, antibiotics which are basic and water soluble have some nephrotoxic potential. Previous reports from this laboratory have dealt with the marked nephrotoxicity of streptothricin antibiotics.[3-5] With streptomycin, which is also basic and water-soluble, anemia due to hypersensitivity to the compound[8] and anemia arising from vascular wall dysfunction,[8] especially in the form of thrombocytopenic purpura, have been reported. Many other antibiotics are known to cause such adverse hematologic reactions, *e.g.* penicillin-G,[9] chloramphenicol[10] and tetracycline.[10] In the previous experiments, administration of streptothricin antibiotics resulted in a marked progressive decrease in the weights of the spleen and liver without any gross or microscopic lesions and in pathologic changes of the kidneys. However, the present study showed no evidence of toxicologic effects of the streptothricin antibiotic on the blood.

The various blood morphological parameters observed failed to reveal any appreciable change in the treated group at any time after administration of the antibiotic, even at 72 hours, when pronounced nephrotoxic effects were demonstrated histopathologically (Table I).

A further study of the effect of streptothricin on erythrocytes by scanning electron microscopy and determination of erythrocytic fragility by the CPC method did not reveal any significant change in the treated group. The scanning electron microscopic observation showed no evidence of deformation of the red cell membrane in rats treated with the streptothricin antibiotic (Fig. 1a—d), though red blood cells exposed to certain drugs are known to undergo morphologic alterations, such as increased biconcavity or biconvexity, eventually becoming spheroidal and ruptured (hemolysis).

No erythrocytic membrane dysfunction at all was observed in the rats dosed with the streptothricin antibiotic when examined by the CPC method, a widely used diagnostic test system which facilitates the detection of changes in the red cell membrane by measurement of dynamic osmotic fragility (Fig. 2 and Table II). The results of the test confirmed that neither erythrocytic membrane dysfunction nor hemolysis occurred in the streptothricin-treated rats.

Thus, it is clear that the streptothricin antibiotic has no toxic effect on the blood. It can be concluded at present that the toxicologic effects of the streptothricin antibiotic are ascribable to its nephrotoxicity (described in the preceding reports), although further detailed investigation is desirable.

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