Hypoprothrombinemia Induced by Gossypol.* (18756)

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Several investigators(1-3) have reported that cottonseed meal or gossypol feeding causes hemorrhages in pigs, rabbits, and rats. In repeated feeding tests in this laboratory hemorrhages occurred in weanling rabbits on rations containing 20% cottonseed meal. Although the hemorrhages were usually evident in the first loop of the small intestine they were sometimes found in the long bones of the legs, in the heart muscle, or around the brain. These observations led to an investigation of the effect of gossypol and cottonseed meal on clotting time, the results of which are reported here.

Methods. Clotting time was determined by a slight modification of the method of Sternberger(4). In this modification thrombinized plasma was diluted to 9.1% of normal plasma. At this dilution and by this procedure the normal clotting time of rabbits and pigs is 8 ± 1 seconds. Gossypol was dissolved in peanut oil for administration by stomach tube to rabbits and pigs except in the direct comparison with Dicumarol when both compounds were dissolved in NaOH and the solutions adjusted to pH 9.4 with HCl; the latter operations were carried out in an atmosphere of N_2. Cottonseed meal containing 0.1% free gossypol was fed at 20% of the ration to weanling rabbits (700-800 g) in 28-day feeding trials. The other components of the basal ration were ground corn or barley 72%, alfalfa meal 7%, and mineral mixture 1%.

Results. The increase in clotting time of rabbits (Fig. 1, Curve A), after administration of gossypol in oil at 3 mg/kg, from 8 to 18 seconds in 16 hours corresponds to a decrease in prothrombin of about 35% as estimated from a dilution curve for normal plasma. Although few pigs are represented, the response under these conditions (Fig. 1, Curve B) was somewhat greater in this species than in rabbits. When compared directly on the same rabbits, gossypol (Fig. 1, Curve C) was somewhat more rapid in action than dicumarol (Fig. 1, Curve D) but the latter induced a greater response of longer duration. The approximately 66% higher molecular weight of gossypol may account for some of the difference between the two substances.

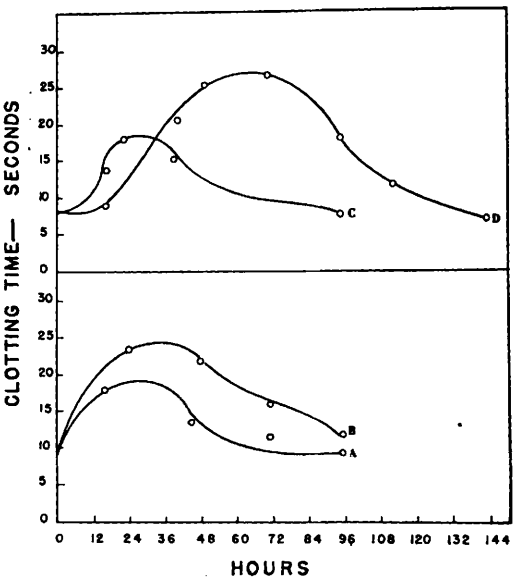

FIG. 1. Effect of gossypol on prothrombin of rabbits and pigs. Administered by stomach tube at 3 mg/kg. Curve A, in peanut oil, avg for 20 rabbits; curve B, in peanut oil, 6 pigs; curve C, in alkaline solution, 6 rabbits; curve D, dicumarol in alkaline solution, 3 mg/kg, same 6 animals as in curve C.

The effect of cottonseed meal feeding on prothrombin of rabbits is illustrated in Fig. 2. This curve is a composite obtained from several 28-day feeding trials with cottonseed meal at the 20% level. In these tests various supplements were added without appreciable effect. The points represent irregular numbers of animals—the first is an average of 96 and the last 58. This drop is due

* Published with approval of the Director of the Ga. Exp. Station as Journal Paper No. 209.

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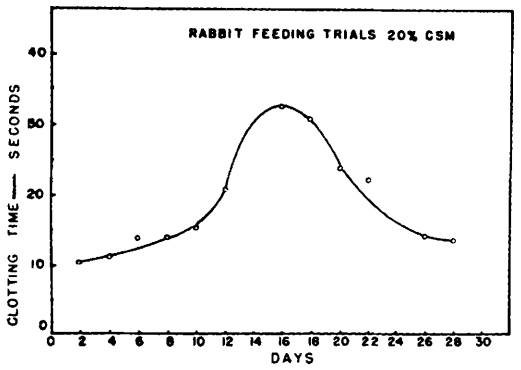

FIG. 2. Effect of cottonseed meal feeding on prothrombin of rabbits. Each point represents avg of preceding and designated day. Avg for over 50 animals at the 2nd, 8th, 12th, 22nd, and 28th days.

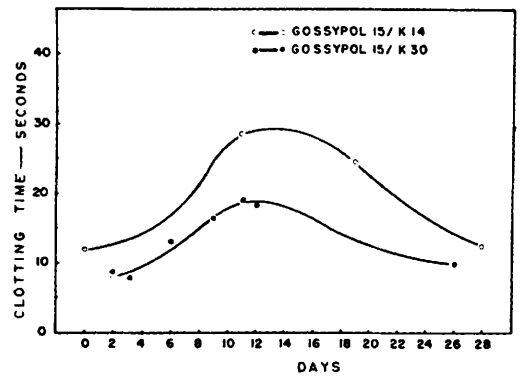

FIG. 3. Effect of vit K supplements on prothrombin of rabbits on 20% cottonseed meal rations. Upper curve; Synkavite added at 14 mg. K/15 mg free gossypol. 8 rabbits. Lower curve; 2-methyl 1,4 naphthoquinone added at 2 mg/1 mg free gossypol. 8 rabbits.

largely to losses, and, after the 14th day this curve more and more shows the response of surviving animals. Most of these rabbits had a calculated intake of about 20 mg of gossypol in the first 2 days; yet the effect on clotting time did not appear until about the 7th day when some of the animals had ingested 70 mg gossypol. This delayed effect is in sharp contrast with that of the direct administration of gossypol at 3 mg/kg. In the feeding tests, of which these were a part, 44 of 103 deaths occurred between the 14th and 21st day when the delay in clotting was around the maximum. However, about 20% of the deaths occurred between the 21st and 28th day of the feeding period and hemorrhages were usually evident even though clotting time approached a normal value. Around the 28th day, the clotting time of 6 animals ranged from 10 to 14 seconds within the 72 hours preceding death. Moreover, 3 animals, which were hemorrhagic, showed clotting time of less than 12 seconds within the 24 hours preceding death which occurred between the 9th and 12th day of the feeding test.

The effect of vit. K supplementation on the hypoprothrombinemia induced in rabbits by the same cottonseed meal is shown in Fig. 3. The upper curve shows that Synkavite, a water soluble form of the vitamin, had no effect on the hypoprothrombinemia induced in rabbits when added to the ration at 14 mg to 15 mg gossypol. The lower curve was obtained when 2-methyl 1,4-naphthoquinone was added to the ration at the rate of 2 mg/1 mg gossypol. Peanut oil was also added to this ration to bring the fat content from about 2.0 to 7.0% Although the added fat might have been a factor there was a decided reduction of the gossypol effect with this form of the vitamin at this very high level. Vitamin K in either of these forms did not prevent hemorrhages or death in these tests.

Discussion. Perhaps the hemorrhagic properties of cottonseed meal have not been emphasized heretofore because hemorrhages are not so apparent in many species or in older animals and their widespread occurrence after cottonseed meal feeding can only be established by histological studies. However, with sensitive young rabbits as the test animal, hemorrhages are more obvious.

The delayed effect of so-called free gossypol in cottonseed meal on clotting time as compared with the relatively rapid action when given directly can hardly be explained on the basis of poor absorption alone. The rather slow increase in clotting time after the inception of the hypoprothrombinemia followed by the slow return toward normal suggests a cyclic response much more complex than might be accounted for by a simple absorption mechanism. The fact that hemorrhages were evident in rabbits which died in the fourth week of the feeding period on 20% cottonseed meal rations when the clotting time approached normal in addition to the reduction of the hypoprothrombinemia by high vitamin

K supplementation without preventing hemorrhages and death, is evidence that hypoprothrombinemia is not the primary cause of hemorrhages and of cottonseed meal toxicity. This affords an interesting parallel to the conclusion of Schofield(5) regarding hemorrhagic sweet clover disease long before Dicumarol was isolated and characterized.

Summary. Gossypol induces hypoprothrombinemia in rabbits and pigs. Cottonseed meal containing free gossypol induces hypoprothrombinemia in young rabbits which begins after about 7 days feeding and passes through a maximum at approximately 17 days then slowly returns to normal around 28 days. Gossypol is not as effective an anticoagulant as Dicumarol but acts somewhat more rapidly.

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Received March 29, 1951. P.S.E.B.M., 1951, v77.

Failure of Aureomycin in Treatment of Experimental Leishmaniasis (18757)

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Aureomycin has recently been demonstrated both experimentally and clinically to be effective against amebiasis(1-4), trichomonas vaginalis vaginitis(5) and malarial infestations(6,7). It is, therefore of interest to know whether this antibiotic will also act on another parasitic infection, namely, Leishmaniasis (*Leishmania donovani*).

Materials and methods. Chinese hamsters, weighing 20 to 30 g each, were infected by intraperitoneal injection of 0.2 ml of a saline suspension of leishmania, prepared by mixing 30 ml of sterile saline solution with a finely ground spleen, freshly removed from a heavily infected hamster. The animals were divided into 2 groups and each group was further divided into 3 subgroups of 20 animals each. Aureomycin* was given intraperitoneally twice a day, *i.e.* 9 a.m. and 5 p.m., for 5 days for group A, for 10 days for group B. For the former, treatment was started immediately after the injection of leishmania, and for the latter, the antibiotic was not given until 2 months after infection. The dosage schedule of aureomycin was carried out according to the following plan:

Subgroup 1. 3 mg per animal per day.

Subgroup 2. 1.5 mg per animal per day

Subgroup 3. 0.5 ml normal saline per animal per day and served as infected, untreated controls.

Results. During the period of treatment, 8 hamsters in group A-1 and 10 in group B-1 died, apparently due to drug intoxication: Post-mortem examination of those animals revealed the presence of hemorrhages in the lungs with extensive peritoneal adhesions. All the rest survived and were killed 2 months after the completion of the treatment. The degree of parasitization was determined by the microscopic examination of the smears made from spleens at autopsy. Results obtained during the present investigation failed

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* The aureomycin used in this study was supplied by Lederle Laboratories Division of American Cyanamid Co., N. Y.