tobarbital. Major results reveal portal hypertension concomitant with systemic hypotension; hemoconcentration; depressed liver function; hepatic, renal and adrenal congestion; intestinal necrosis and gall bladder edema. The liver appears to be the primary "target organ" of endotoxin in regard to hemodynamic, histological, and functional abnormalities. The coyote is more resistant to endotoxin than its domestic counterpart in terms of changes in hemodynamics and survival ability.

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## Effect of Dietary Factors on Prothrombin Response to Acenocoumarin in Guinea Pigs.* (32069)

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The influence of dietary factors and gastrointestinal disturbances on the prothrombin response to coumarin anticoagulants has often been reported(1). Relatively short periods of starvation can induce significant exaggerations in the response to these agents (2). Whether this effect is a function of low caloric intake, or an acute deficiency in a specific nutritional component is not known. The present study compares the effects of a balanced diet, starvation and diets free from carbohydrate or protein on the prothrombin response to the anticoagulant, acenocoumarin, in guinea pigs. The data indicate that modest amounts of dietary protein will prevent the exaggerated response resulting from short periods of starvation, whereas an equivalent amount of carbohydrate will not.

*Methods.* Non-albino male guinea pigs weighing 250-350 g were fed Rockland Guinea Pig diet (Tekland Inc., Monmouth, Ill.) supplemented with lettuce and water *ad libitum* until initiation of the experiment. Each animal consumed 25 to 40 g per day. On experimental days 1 through 3, feeding was confined to the pattern described below. Special mixes were prepared by General Biochemicals, Chagrin Falls, Ohio, and contained 15% nonnutritive fiber, 7.3% corn oil, and 9.3% salts, vitamins, and minerals. The carbohydrate mix included 15.0% corn starch, 10.3% sucrose, and 42.8% glucose, while the protein mix included 68.1% casein. Diets were fed by hand in the form of 10 capsules (Parke, Davis No. 3) twice a day, each containing 200 mg of pulverized food (total of 4 g per day) and was regularly observed to be

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TABLE I. Effect of Diet on Response of Guinea Pigs to Acenocoumarin.*

| Diet | No. of animals | Mean prothrombin time (sec) | Standard error |
|---|---|---|---|
| Normal | 16 | 100.9 | 4.78 |
| Starvation | 17 | 126.7 | 6.71 |
| Carbohydrate | 16 | 117.2 | 5.40 |
| Protein | 14 | 102.6 | 5.07 |

* The prothrombin time of normal untreated guinea pigs by the method used is about 36 sec(2).

consumed in toto. Acenocoumarin (25 mg/kg i.p.) was administered on days 2 and 3, and animals were sacrificed on the morning of day 4. Cardiac blood specimens were obtained and studied for clotting activity by methods previously described(2).

*Experiment I.* The effect of 4 diets was compared: 1) regular diet, 2) starved, 3) carbohydrate diet (protein-free), and 4) protein diet (carbohydrate-free). Two or three guinea pigs in each of the 4 groups were studied in parallel each week for 7 weeks until the prothrombin response was determined in a total of 14 to 17 animals per group after 7 sets of studies. The data were subjected to analysis of variance, and the differences among all group means were tested by Tukey's(3) method for multiple comparisons.

*Experiment II.* In a similar design to Experiment I, the prothrombin response to acenocoumarin was compared in totally starved animals *vs* animals fed 10 empty capsules twice a day (total capsule weight 0.75 g/day). Two studies with 5 animals per group per study were performed.

*Experiment III.* The electrophoretic patterns(4) of serum of 5 animals in pH 8.3 barbital buffer were studied before and 48 hours after starvation.

*Results.* I. Table I presents the mean and standard error of the prothrombin time of the 4 groups. Table II presents the probability of significance of the differences between means as calculated by Tukey's "t" test. It is apparent that the high protein diet animals are not significantly different from the normal diet group, but both of these showed significantly less prothrombin response than the starved and protein-free group.

II. A comparison of starved animals with those fed 20 empty gelatin capsules per day yielded an average prothrombin time of 124.4 seconds for the completely starved group, and 135.0 for the group fed empty capsules, indicating that the capsules *per se* do not reduce the exaggerated prothrombin response caused by acute starvation.

TABLE II. Probability of Significance of Difference Between Means.

| | Difference between means (sec) | P value | Tukey's "t"(3) |
|---|---|---|---|
| Normal *vs* starvation | 25.7 | <.01 | 25.7 |
| Normal *vs* protein | 1.6 | .8 | 1.7 |
| Normal *vs* carbohydrate | 16.3 | .018 | 16.3 |
| Starvation *vs* carbohydrate | 9.5 | .3 | 9.4 |
| Starvation *vs* protein | 24.1 | <.01 | 24.0 |
| Carbohydrate *vs* protein | 14.6 | .05 | 14.6 |

III. The electrophoretic patterns of serum before and after 48 hours of starvation showed essentially no differences in the 5 animals studied.

*Discussion and conclusions.* The data presented confirm previous reports(2) that short periods of starvation result in a significant enhancement in the prothrombin response to a standard dose of a coumarin anticoagulant. The present data demonstrate that a limited amount of protein prevents the hypersensitivity of starvation, whereas equivalent caloric intake in the form of carbohydrate does not. Although short periods of starvation *per se* do not produce a detectable deficiency in circulating prothrombin(2) or serum proteins as detected by electrophoresis, coumarin inhibition of prothrombin synthesis is measurably exaggerated by dietary protein deficiency. Reduced availability of prothrombin substrate derived from dietary protein is one possible responsible factor.

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## Effect of Asiaticoside on Wound Healing in the Rat. (32070)

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The mechanisms of wound healing and the agents which promote wound healing have received considerable attention in recent years (1-3). Prudden and co-workers(4,5) reported that powders prepared by acid-pepsin digestion of bovine tracheal cartilage effectively increase the tensile strength of wounds in experimental animals and humans and promote healing of chronic wounds in humans. Another healing agent reported as effective(6,7) is Asiaticoside,* a triterpene glycoside obtained from *Centella asiatica*.

The work reported here was performed with Asiaticoside. All results were obtained by measuring the tensile strength (tension for disruption) of healing linear wounds in rat skin.

*Methods.* The method of Fenton and West (8) with some modification, was used in the experiments. Male rats (Charles River C-D 1 strain) were selected that ranged in weight from 222 to 264 g; however, the range on any one day of testing was usually narrower.

Under ether anesthesia, the back of each rat was close-clipped and a midline incision 4 cm long was made through the whole skin thickness with a sharp scalpel. Each wound was closed with 3 equally spaced interrupted sutures of 4-0 silk. On the sixth day post wounding, each rat was sacrificed with sodium pentobarbital and a strip of skin 20 × 60 mm, with the wound in the middle, was excised and placed on a horizontal board. Skin on one side of the wound was firmly fastened with pins to the board. On the other side, a hook was passed through the skin at a point opposite the center of the wound. A string tied to this hook was passed horizontally over a pulley and thence downward to a suspending platform. The wound sutures were then removed, a plastic bottle placed on the platform, and mercury run into the bottle at a constant rate until the wound disrupted. The mercury was run from a 125 ml leveling bulb by way of a tube fitted with a one way stopcock and a 19 gauge hypodermic needle. The head was 25 cm and rate of flow was 370 g/minute. The total weight of mercury, container and platform in g, divided by length of wound in cm, gave a tensile strength value of g/cm wound.

The wounds were medicated with one local application (5-10 mg) of Asiaticoside, or a dilution of the glycoside, before being sutured. Dilutions were made by adjusting the undiluted glycoside with the 2% powder.

Three groups of 10 rats each were used at one time. One group with no medication served as control and each of the other 2 groups was treated with 2 different concentrations of the medicament.

The sixth day was selected for tensile strength testing because preliminary experiments had shown this to be a good time to detect either decreased or increased strength resulting from drug treatment.

On occasion a wound was observed to be infected; such wounds were discarded. No cultures or histologic sections were made.

*Results.* Table I shows the results obtained. The undiluted Asiaticoside gave statistically significant increases of tensile strength in 7 of 8 test groups. When the glycoside was diluted to 50%, 3 of 4 tests were positive, the increases being statistically significant. Con-

* 2$\alpha$, 3$\beta$, 23-Trihydroxy-12-ursen-28-oate of 1-[*O*-$\alpha$-*L*-rhamnopyranosyl (1→4)-*O*-$\beta$-D-glucopyranosyl (1→6)]*O*-$\beta$-glucopyranose. Supplied to us through the courtesy of Laboratories Laroche Navarron, Paris, as undiluted Madecassol and as the 2% powder.