

Chemical Studies in Experimental Scorbutus in Cortisone-Treated Guinea Pigs.* (20964)

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In a previous communication the evidence that cortisone had no effect upon the sequence of events in both the production of and early repair from experimental scurvy in guinea pigs, using gross and histological changes as criteria, was documented(1). It has been suggested that depolymerization of ground substance, one of the components of intercellular substances, may occur in scurvy(2), and that the elevation of serum glycoprotein values in the scorbutic guinea pig, a fact established by the work of Pirani and Catchpole(3) may be a direct result of this depolymerization mechanism. If this is so, the action of cortisone upon scurvy might very well be reflected by alterations in these heightened values. If no effect is demonstrated, this would be compatible with the histological findings cited above. With these ideas in mind this study was initiated. It reports the serum mucoprotein values, as well as serum cholesterol and lecithin levels of scorbutic guinea pigs treated with cortisone.

Experimental. Forty-two guinea pigs were employed in this study, divided as indicated in Table I. In the scurvy plus "prophylactic" cortisone, cortisone was begun at the same time as the guinea pigs were placed on the

scorbutic diet. Cortisone was given intraperitoneally at the following levels; to one animal 10 mg cortisone per kg of body weight, to two others 5 mg cortisone per kg of body weight. In the scurvy plus "curative" cortisone group, the guinea pigs were allowed to progress to the symptom stage before cortisone was given. Again this was administered intraperitoneally at the following levels—one at 10, two at 5, and one at 2.5 mg cortisone per kg of body weight. Two repair periods were selected in the subsequent categories, 72 and 96 hours respectively, during which time 50 mg of ascorbic acid per day was given by mouth to every animal. Guinea pigs were studied during these periods with and without cortisone administered intraperitoneally at the following levels, 2.5 and 5.0 mg cortisone per kg of body weight. In the prophylactic and curative cortisone groups the animals ranged in weight initially from 120 to 201 g, while in the repair group they were somewhat heavier, weighing between 248 and 300 g. Five additional untreated scorbutic animals, averaging 400 g in weight were used to determine mucoprotein tyrosine values. In both untreated scurvy and in control groups, intermediate weights were encountered. The diet used for produc-

TABLE I.
Mucoprotein Tyrosine and Glucosamine in Scorbutic Guinea Pigs with and without Cortisone.

	No. of animals	Avg duration of exp., days	—Serum—	
			Mucoprotein, tyrosine, mg % $\pm$ S.D.	Glucosamine, mg % $\pm$ S.D.
Scurvy	13	23.8	9.47 $\pm$.088	93.25(8)* $\pm$ 1.177
" + "prophylactic" cortisone	3	28.3	9.96 $\pm$.264	95.76 $\pm$ 6.474
" + "curative" "	4	25.8	9.70 $\pm$.260	86.20(3)* $\pm$.5102
Scurvy + repair—72 hr	2	26.0	9.55 $\pm$.177	94.60 $\pm$ 1.929
idem —96 "	4	24.0	9.30 $\pm$.210	101.60 $\pm$.975
Scurvy + cortisone + repair—72 hr	2	26.0	8.50 $\pm$.638	102.75 $\pm$ 1.241
idem —96 "	4	24.0	7.96 $\pm$.167	97.63 $\pm$ 2.355
Controls	6	27.4	5.17 $\pm$.190	74.05 $\pm$ 2.780

* No. in parentheses indicate number of animals used to determine avg for value in question.

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TABLE II. Cholesterol and Lecithin in Scorbatic Guinea Pigs with and without Cortisone.

	No. of animals	Avg duration of exp., days	Serum		Ratio chol./lecith.
			Cholesterol, mg % $\pm$ S.D.	Lecithin, mg % $\pm$ S.D.	
Scurvy	11	23.3	92.18 $\pm$ 2.71	168.02(8)* $\pm$ 9.87	.548
" + "prophylactic" cortisone	2	26.5	99.00 $\pm$ 10.28	122.00(3) $\pm$ 14.45	.812
" + "curative" "	6	24.2	117.45 $\pm$ 6.33	111.50(2) $\pm$ 3.18	1.052
Scurvy + repair—72 hr	3	26.7	109.46 $\pm$ 11.04	165.50(2) $\pm$ 22.97	.662
idem —96 "	4	24.0	188.75 $\pm$ 4.98	203.30 $\pm$ 5.60	.914
Scurvy + cortisone + repair—72 hr	5	27.4	127.46 $\pm$ 8.97	172.00(2) $\pm$ 9.22	.742
idem —96 "	4	24.0	194.75 $\pm$ 13.95	194.25 $\pm$ 10.60	1.002
Controls	23	—	52.20 $\pm$ 0.51	62.46 $\pm$.75	.835

* No. in parentheses indicate number of animals used to determine avg for value in question.

ing the deficiency was ground Purina rat chow (checkers) heated overnight at 100°C. with 4% dried yeast added, and then mixed with sufficient water to moisten and thus make it more acceptable to the guinea pigs. One cc of cod liver oil was given twice weekly. This diet, supplemented with ascorbic acid (50 mg by dropper daily) was adequate for maintenance of good health in the control animals. The guinea pigs were kept in individual wire bottom cages, allowed food and water *ad libitum* and weighed daily. The duration of the experiment varied between 20 and 30 days, being determined in the "cortisone prophylactic" and "cortisone curative" group by the condition of the animals which were killed in the very late stages of scurvy. Scurvy controls and diet controls were killed at corresponding times so as to furnish adequate material for comparative tests. Bleeding was done by cardiac puncture under light ether anesthesia. Bloods were analyzed for total cholesterol by the Liebermann-Burchard method, for lipid-P, by the method of Fiske and SubbaRow, for mucoprotein tyrosine by Winzler's method(4),[†] and for serum glucosamine by a modification of Boas(5) of Elson and Morgan's method(6).

Results. The results are presented in tabular form. From Table I, one may deduce that cortisone, administered either "prophylactically" or "curatively" has no influence upon the level of mucoprotein tyrosine values in serum from scorbatic guinea pigs. Cortis-

one, administered together with ascorbic acid, appears to hasten the return toward normal values as compared to the results seen in guinea pigs recovering from scurvy with the aid of ascorbic acid alone. The glucosamine values, always considered less reliable because of the inherent difficulties in the procedure, show no downward trend in repair by cortisone and ascorbic acid.

Cholesterol and lecithin values, elevated in scurvy, are even higher when cortisone is administered to scorbatic animals (Table II). In early repair, also, the values are higher and rise with duration of repair, at least up to 96 hours, the longest time span studied. Cortisone thus further elevated the high values of the two lipid components studied in early repair.

Discussion. The results obtained were probably to be anticipated. Cortisone given to scorbatic animals does not in any way influence the course of the deficiency, the histology, or that part of the chemical picture that reflects the deficiency syndrome. In repair, mucoprotein tyrosine values tend to decline, a process that appears to be accelerated by the simultaneous administration of cortisone. This phenomenon is not reflected in the glucosamine values. We have no explanation for this except to say that there is no reason to expect an exact correspondence between these two sets of values, since the procedures employ different serum protein moieties. More reliance should be placed on the mucoprotein tyrosine results because of the greater accuracy and reproducibility of the method.

The higher cholesterol and lecithin values in cortisone "modified" scurvy may merely

[†] A slight modification in procedure, *viz*, dilution of the blood serum with physiological saline, 1:1, before addition of the HClO₄ precipitating reagent, was used.

reflect a summation of the scurvy and the cortisone effect(7,8) on these particular lipid components of blood serum.

Summary. 1. Sera of guinea pigs suffering from uncomplicated scurvy and sera of scorbutic guinea pigs where cortisone was administered, do not differ from one another as regards the following values: mucoprotein determined as a) tyrosine; b) glucosamine. 2. Sera of scorbutic guinea pigs recovering from the deficiency through the administration of ascorbic acid approach normal mucoprotein tyrosine values more rapidly when cortisone is administered concurrently with ascorbic acid. 3. The administration of cortisone elevates the already high cholesterol and lecithin values in scorbutic guinea pigs.

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Effect of Cortisone upon Local Capillary Permeability. (20965)

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Among the important effects of the cortical steroids is their influence upon endothelium. Kendall(1) gave as their primary function, the control of permeability of cells, and of the transfer of inorganic ions and water between the cells and the extracellular space. The control of permeability applies to endothelium as well as to other cells. Physiologists (Best and Taylor(2)) agree that one property of cortisone is to maintain the impermeability of capillary endothelium. Ebert(3) and Lurie (4) reported that cortisone had this effect in reactions to injury; Balourdas and Chambers (5) found that cortisone caused reduction of capillary leakage in the exteriorized meso-appendix of rats. Whitelaw(6) used ACTH by injection in the treatment of burned patients and reported a definite decrease in the oozing from burned surfaces. Other such observations might be cited.

A simple experiment was devised to produce additional evidence upon the effects of cortisone upon capillary permeability. It is well known that trypan blue will cause staining in areas of increased permeability seen in in-

flamed or injured tissues. The test consists of injecting a solution of trypan blue intravenously, then applying to the skin the agent to be tested. The prompt development of a blue zone about the test point indicates that the capillaries there are abnormally permeable. We(7) have shown that extracts of normal tissues and various substances containing products of protein cleavage, will cause a local increase in permeability if injected into the skin.

Methods. A weighed amount of tissue was suspended finely in 4 volumes of 0.9% NaCl solution in a Waring Blendor. This was centrifuged, the supernatant fluid was adjusted to pH 7 and sterilized by filtration (Seitz). Solutions of substances containing products of protein digestion were sterilized in the same manner. These included Neo-peptone, Bactoprotone (each by Difco), Witte's peptone and a peptic digest of fibrin made in our laboratory. A series of dilutions was tested to determine what amounts would produce a positive trypan blue reaction. Such amounts of each extract and solution were tested in dupli-