

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.

1. Wolbach, S. B., and Maddock, C. L., *Arch. Path.*, 1952, v53, 54.
2. Gersh, I., and Catchpole, H. R., *Am. J. Anat.*, 1950, v85, 457.
3. Pirani, C. L., and Catchpole, H. R., *Arch Path.*, 1951, v51, 597.
4. Winzler, R. S., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, 1948, v27, 609.
5. Boas, Norman F., Personal communication to Paul R. Patterson.
6. Elson, L. A., and Morgan, W. T., *Biochem. J.*, 1933, v27, 1824.
7. Adlersberg, D., Schaefer, L. E., and Dritch, R., *J. Clin. Invest.*, 1950, v29, 795.
8. ———, *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 877.

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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-

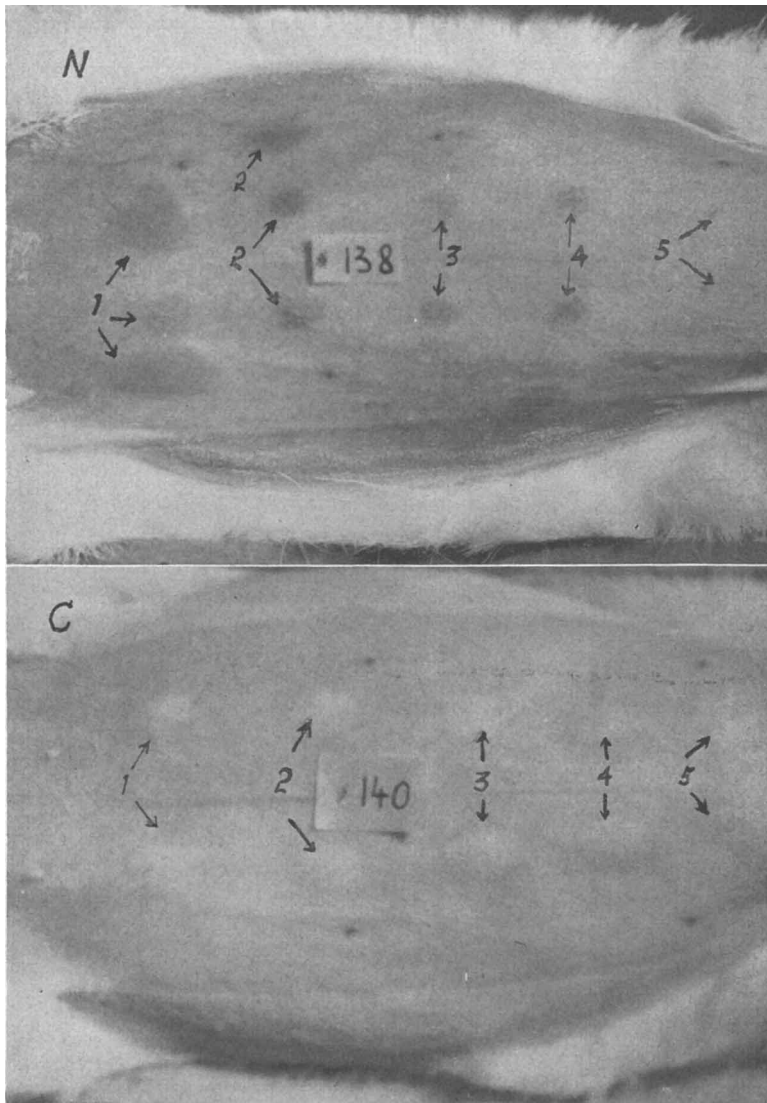


FIG. 1. Photograph of permeability tests. N indicates normal (#138); C indicates cortisone treated animal (#140). 1: histamine, 0.01%; 2: fibrin, peptic digest, 0.05%; 3: Neopeptone, 0.05%; 4: Bacto-protone, 0.05%; 5: NaCl solution, 0.9%, control.

cate on the same animal in each experiment. A 0.01% solution of histamine was used as a positive control, and a 0.9% solution of NaCl as a negative control. Healthy male New Zealand white rabbits were used. The fur over the abdomen was sheared closely with clippers, using care not to injure the skin. Then a freshly made 1% solution of trypan blue was injected intravenously, 1.0 ml per kg. An injection of 0.1 ml of the solution to be tested was made into the skin with a

26-gauge needle. Rabbits were used in pairs, one having received intramuscular injections of cortisone 18 hours and one hour before the injections; each dose contained 5 mg/kg of cortisone. The same agents in identical doses were injected into a control animal which had received no cortisone (Fig. 1). Very strong reactions were recorded as 3+, strong 2+, moderate 1+, doubtful $\pm$, and negative —. The findings are given below in tabular form.

Results. It is observed that the results

TABLE I. Digests of Protein.

Substance	Dilution	Tests	3+	2+	1+	±	—	
Neo-peptone	.0005	{ N*	12	3	2	4	1	2
		{ C*	14	0	0	0	0	14
Bacto-protone	.0005	{ N	14	6	8	0	0	0
		{ C	14	0	0	0	3	11
Fibrin digest	.0005	{ N	14	6	4	4	0	0
		{ C	14	0	0	1	2	11
Witte's peptone	.0005	{ N	6	6	0	0	0	0
		{ C	6	0	0	2	0	4
Histamine	.0001	{ N	10	5	3	2	0	0
		{ C	10	0	0	0	2	8

* N indicates normal; C indicates cortisone treated animals.

varied somewhat even when the agents, the dilutions and the dosages were identical. A minor factor in this may have been a difference in the responses of individual animals. But it is noted that the variations were much greater in the reactions to extracts of tissues than to the products of protein digestion. We regard it as significant that the reactions to histamine were quite uniform. Now histamine is a chemical entity whose composition is known. Some have assumed that extracts of tissues produce increased capillary permeability because they contain a chemical entity—"leukotoxine". If this were true, then the extracts should evoke a more uniform response as do histamine and certain digests of protein.

The tabulated results indicate that cortisone, given intramuscularly prior to experimentation, tends to maintain the impermeability of endothelium against various agents which produce capillary permeability in untreated animals. This finding does not coincide with that of Shleser and Freed(8), who reported that Compound E (cortisone) when injected intradermally together with a 2% solution of Bacto-peptone, did not prevent capillary permeability. Menkin(9) reported that cortisone acetate alone, injected intradermally, induced a rapid and constant increase in capillary permeability as shown by the trypan blue test. The dose injected into the skin may differ from the effect of the same hormone introduced systemically.

Specimens of skin in test areas were sectioned and stained routinely for histologic examination. Some were taken one hour after the test, others after 2.5 hours. The lesions

from the untreated animals regularly showed acute edema and beginning leukocytic infiltration after one hour; these features were more advanced 2.5 hours after the injections. The acute inflammation was most pronounced in the lesions produced by Bacto-protone, peptic digest, and by extracts of spleen and of lung. Both edema and leukocytic infiltration were of lesser degree in the cortisone-treated rabbits. This finding confirms our previous report(10) and the observations of others that cortisone inhibits the development of acute inflammation. One observation was of special interest: in the untreated animals, the leukocytes had migrated freely from the minute vessels into the tissue spaces. In contrast, in the cortisone-treated animals, the leukocytes came to the area but remained mostly within the lumina of the capillaries and

TABLE II. Extracts of Tissue.

Substance	Dilution	Tests	3+	2+	1+	±	—
Kidney	1/8	{ N*	9	8	1	0	0
		{ C*	6	0	2	2	0
"	1/16	{ N	12	8	4	0	0
		{ C	8	0	2	4	0
"	1/64	{ N	5	2	2	1	0
		{ C	5	0	0	0	5
"	1/128	{ N	4	2	0	2	0
		{ C	4	0	0	0	4
Lung	1/8	{ N	4	4	0	0	0
		{ C	4	0	0	2	1
"	1/16	{ N	6	2	2	2	0
		{ C	4	0	0	0	2
Spleen	1/2	{ N	6	6	0	0	0
		{ C	4	0	0	2	0
"	1/4	{ N	6	2	3	1	0
		{ C	4	0	0	0	2

* N indicates normal; C indicates cortisone treated animals.

venules; relatively few had migrated into the tissue spaces. This observation suggests that leukocytes migrate more readily through the walls of capillaries whose permeability has been increased. The capacity of cortisone to maintain endothelial impermeability is probably an important factor in its retarding effect upon acute inflammation.

Summary. 1. Various products of protein digestion and extracts of normal tissues caused increased capillary permeability when injected intradermally. This effect was reduced greatly by pretreatment with cortisone. 2. Local edema and leukocytic infiltration developed at the site of the injections. These effects were more acute in the untreated animals. There was evidence that cortisone inhibited the emigration of leukocytes from within the minute vessels.

1. Kendall, E. C., *The Adrenal Cortex. Arch Path.*, 1941, v32, 474.
2. Best, C. H., and Taylor, N. B., *The Physiologic Basis for Medical Practice*, Baltimore, Williams and Wilkins Co., ed. 5, 1950, p313.
3. Ebert, R. H., *J. Clin. Invest.*, 1951, v30, 636.
4. Lurie, M. B., Zappasodi, P., Dannenberg, A. M., Jr., and Lynch, E. C., Abstr., 47th Ann. Meet., *Nat. Tuberc. Assn.*, May 14, 1951, p4-5.
5. Balourdas, T., and Chambers, R., *Fed. Proc.*, 1952, v11, 8.
6. Whitelaw, M. J., Proc. 2nd Clin. ACTH Conf., Philadelphia, Blakiston, 1951, p310.
7. Moon, V. H., and Tershakovec, Geo. A., *A. M. A. Arch. Path.*, 1953, v55, 384.
8. Shleser, I. H., and Freed, S. C., *Am. J. Physiol.*, 1942, v137, 426.
9. Menkin, V., *Am. J. Physiol.*, 1951, v165, 294.
10. Moon, V. H., and Tershakovec, Geo. A., *Proc. Soc. Exp. Biol. and Med.*, 1952, v79, 63.

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Curare-Like Activity of Some Bis-Fluorenyl-Bis-Quaternary Ammonium Compounds. (20966)

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Bovet(1) has proposed a very interesting physical distinction between those agents which induce myoneural junction blockade by either depolarization or stabilization of the end-plate. He proposes the term "leptocurare" for agents which induce blockade by depolarization and for which the molecular configuration usually is long and thin, *i.e.*, decamethonium. "Pachycurare" is proposed for those agents which induce blockade by stabilization of the membrane, such as d-tubocurarine, and for which the molecular configuration is quite bulky in width. In the reports of Barlow and Ing(2,3), Paton and Zaimis(4,5) and in the excellent reviews of Paton(6) and Riker(7), it has been observed that maximum curariform activity, with bis-quaternaries, is obtained when the distance between quaternary nitrogen atoms is approximately 15 Å, irrespective of the type of blockade instituted.

This is a report on the neuromuscular blocking properties of a new series of compounds prepared by Gray *et al.*(8), in which the nitrogen atoms are joined by a polymethylene chain and in which one of the groups on each nitrogen atom is a fluorenyl group. The compounds are of interest in that the large terminal groups modify the distance between nitrogen atoms which have been reported necessary for optimal activity. A similar modification of activity by terminal groupings was reported by Wien *et al.*(9) for the ganglionic blocking agents in the methonium series.

Methods. 1) *Mice.* The inclined screen procedure of Thompson(10), as modified by Hoppe(11), was used for the quantitative determination of the potency of these compounds. Concentrations were varied by equal increments so that at least 3 reactive groups were obtained between the ED₀ and the