TABLE I. Effect of Irradiation with Unfiltered Light from a CH-4 Mercury Lamp on Excretion of Urinary Coproporphyrin in Lead-Poisoned Rabbits. Urinary coproporphyrin in μg/24 hr.

Day	R18	R21	R23
10		250	210
11		275*	220*
12		950	550
13			520
14		480	480
15	110	450*	350*
16	150*	650	655
17	650	550	400
18	330	470	380
19	180	270	360
20	250	270	250
21	110	273	280*
22	160	150	360
23	80	80	200
24	110	82*	
25	180	200	
26	170*	90	
27	350	88	
28	390	88	
29		60	
30	150	60	
31	150*	50	
32	180	50	
33	210	50	
34	110		

* UV light exposure on day indicated. Lead given on day 0 in each instance. No analyses were made on days 1-14 on rabbit R18 and days 1-10 on rabbits R21 and 23.

ing, (2) that the light stimulates or brings about a rapid increase in coproporphyrin formation. The observation (Table I) of diminishing effect of repeated ultra-violet exposures, is compatible with either possibility, as in the first instance it could be regarded as exhaustion of stored porphyrin, and in the second, of the cellular potentiality to make the excessive porphyrin. In a recent study of the urinary coproporphyrin in lead poisoned rabbits, carried out with the aid of glycine containing N^{15}(5) the findings indicated that the increased porphyrin was more likely formed as a result of the lead, rather than a heightened excretion of preformed porphyrin. In the present study, however, it is entirely conceivable that the effect of light is to increase the excretion of preformed porphyrin.

Summary and conclusions. 1. Light exposure increases the urinary coproporphyrin excretion in rabbits with lead poisoning. 2. The increase is especially marked after ultraviolet radiation. Subsequent radiation, however, fails to cause a renewed rise or is followed by only a small increase. 3. Whether the increase following light is due to mobilization of preformed porphyrin, or to increased porphyrin formation, has not been determined.

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Sulfhydryl Inhibition as a Mechanism in the Effects of ACTH and Cortisone.* (18643)

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Suppression or partial inhibition of cellular enzyme systems seems to be at least one widespread mechanism of action of the C_{11}-oxygenated adrenal-cortical steroids. The demonstrated disrupting effect(1) of these steroids on phases of carbohydrate metabolism may well be mediated thru inhibition of enzyme systems essential to the normal processing of glucose by the organism. If such inhibitory effect is as widespread as is suggested, it

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would seem reasonable to anticipate the presence of some common denominator of mechanism in the systems affected. It has been demonstrated that ACTH reduces availability of sulfhydryls(2) to the organism, a deprivation which in obligate sulfhydryl enzyme systems could effectively block enzymatic activity(3). Carboxylase, phosphoglyceraldehyde oxidase, phosphoglucomutase, pyruvate oxidase, adenosinetriphosphatase are all enzyme systems allegedly dependent on –SH groups for their activity(3). Those enzyme systems of the tricarboxylic acid cycle which depend on Coenzyme A for their functional integrity(4) all require sulfhydryls, since Coenzyme A is itself a sulfhydryl obligate. Besides the C_{11}-oxygenated adrenalcortical steroids, other agencies are known to rob the metabolic pool of its sulfhydryl groups by the oxidation of –SH to SS. Notable among these is ascorbic acid(5) thru its oxidation product, dehydroascorbic acid, which oxidizes –SH to SS.

It seemed feasible first to study in parallelism the effects of cortisone and of massive administration of ascorbic acid on the sulfhydryl content of human whole-blood.

To establish base-lines and at the same time to test methodology, whole-blood levels of sulfhydryls and of ascorbic acid were determined in 20 normal individuals, using the method of Woodward and Fry(6) for –SH and the indophenol method of Jung for ascorbic acid(7) making the necessary quantitative –SH corrections for the reducing effects of the ascorbic acid present. The results of these determinations were found to fall well within the range of expectancy. Massive doses of ascorbic acid (up to 2 g by vein) were then rapidly administered to 8 of these normal individuals, repeating the same blood determinations before and after the ascorbic acid administration. The same procedure was then carried out in 5 patients with typical rheumatoid arthritis who had previously shown on one or more occasions temporarily striking but evanescent clinical improvement on massive doses of ascorbic acid administered by vein (without DOCA). In addition, whole-blood sulfhydryl determinations were made in 5 other rheumatoid arthritics before and after completely effective therapy with cortisone.

Findings. The "before and after" –SH readings on the whole blood of all 3 groups were too variable and small in their differences to be experimentally significant. The negative findings on humoral (blood) assay for sulfhydryls after administration of known –SH depletors suggested that the blood might in no wise reflect a dearth or sufficiency of these groups in the cells themselves, where, after all, the intracellular enzyme systems would be functioning and where, accordingly,

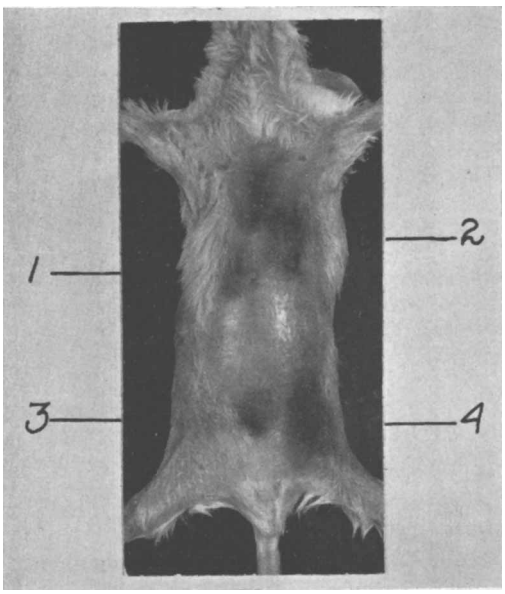

FIG. 1.
1—Saline-India ink control.
2—Saline-hyaluronidase-India ink control.
3—Hyaluronidase after cortisone.
4—Hyaluronidase after G-SH.

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conversions from –SH to SS would be registered by failure of these systems. To shed further light on sulfhydryl relation to enzyme function in closer proximity to fixed tissue cells, the skin-spreading activity of the hyaluronidase enzyme system was explored. This system was chosen since it had been shown by Opsahl(8) and by Seifter(9) to be at least partially inhibited by adrenal cortical hormone *in vivo* as well as *in vitro* and since the spreading effect of hyaluronidase in skin would lend itself to rough quantitative visual appraisal of activity and reversibility of such activity.

Procedure. Depilated skin of white mice was used as a proving ground for the influence of free sulfhydryls (reduced glutathione) in reversing the known inhibitory effect of cortisone on the skin-spreading phenomena of mixtures of hyaluronidase in India ink, using the skin technic of Opsahl(8). A group of 18 white mice averaging 15 to 20 g in weight were prepared by depilation of the skin of the abdomen and were kept on a high-protein diet for 3 days to insure against primary depletion of –SH groups. As a control 0.1 cc of normal saline marked with India ink was injected intracutaneously in the right upper quadrant. As a further control, 0.1 cc of saline containing 75 turbidity units of commercial hyaluronidase per cc and marked with India ink was injected intracutaneously into the left upper quadrant. The mice were then prepared further by the subcutaneous injection of 1 mg of cortisone acetate given twice at twelve-hour intervals. Six hours after the second dose, 0.1 cc of normal saline containing 75 turbidity units of hyaluronidase per cc marked with India ink was injected into the skin of the right lower quadrant. After an interval of one-half hour each animal was given 40 mg of reduced glutathione in saline solution intraperitoneally. One-half hour later, 0.1 cc of hyaluronidase in saline was injected into the left lower quadrant intradermally. The spread of the India ink in the skin was measured in 15 minutes and again after 24 hours.

RESULTS

Skin tests	Total	Diameter of spreads 0.5 cm	1.0 + cm	1.5 + cm
1. Saline control	18	12	6	
2. Hyal. control	18		13	5
3. Hyal. after cortisone	18	13	5	
4. Hyal. after G-SH	18		8	10

Negative findings in 3 animals (or Spreads 2, 3, and 4 are equal).

In 15 of the 18 mice, the original or an increased skin-spread was re-established, presumably thru the de-inhibiting effect of reduced glutathione in cortisone-inhibited animals.

In a control group of mice the procedure was repeated, substituting oxidized glutathione (G-SS-G) for G-SH. There was no appreciable change in the cortisone-inhibited hyaluronidase spreading phenomenon in these animals.

Conclusion. Evidence has been presented to suggest that ACTH and cortisone produce their inhibitory action on the skin-spreading effect of the hyaluronidase enzyme system thru the agency of sulfhydryl deprivation. This finding warrants further exploration of enzyme systems known to be inhibited or depressed by the C_{11}-oxygenated adrenal-cortical steroids for sulfhydryl deficiency created by these steroids as a common denominator in the inhibitory effect. The humoral (whole-blood) content of –SH groups after clinically effective therapy with cortisone does not necessarily reflect a sufficiency of this factor in the fixed tissues where its influence on the intracellular enzyme systems would be registered by the functional integrity of these systems.

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