there is, in addition, a significant drop in levels of β- and γ-globulins. Reductions in serum levels of albumin, α_1-globulin and γ-globulin in combined deficiency of Vit. B_{12} and PGA are greater than those in single deficiencies of either vitamin. 2. In Vit. B_{12} deficiency, an increase in proportion of β-globulin is attended by increase in relative concentration of free Vit. B_{12} in serum. It is suggested that there may occur a preferential synthesis of β-globulin in the deficient state whereby mobilization of the available vitamin may be effected. 3. The observed alterations in serum level of γ-globulin are similar to the known effects of corresponding vitamin deficiencies on antibody synthesizing ability of the animal.

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Some Systemic Chemical Responses to Local Inflammation.* (24141)

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Dunphy and Udupa(1) have recently described chemical alterations in rat skin during wound healing. Using simplified micromethods(2), we studied the effect of inflammation on hexosamine, nitrogen and hydroxyproline concentration of both necrotic and normal skin of rats injected intradermally with croton oil to produce localized injury.

Materials and methods. Wistar strain male rats weighing 200 to 300 g were used. Appropriate areas of shaved skin were removed from animals killed by etherization, dissected clean of adhering fat and muscle and stored in frozen state until analysis. The tissue was minced, and autoclaved 3 hours with 10 ml/g 4N HCl. Subsequent analytical procedures have been described(2). The results are expressed in terms of μmoles of hexosamine or hydroxyproline/mmole nitrogen. To provide controlled areas of necrosis, 0.4 ml croton oil (Magnus, Mabee and Reynard Co. N. Y.) was injected intradermally into a previously shaved area of rat skin. The preparation gave no gross evidence of wound healing for at least 7 days, but produced necrosis throughout an area of about one cm^2 within 3 days. The injury included dermis, epidermis and subcutaneous tissue with no suppuration and minimal sloughing. To demonstrate relative homogeneity of the chemistry of rat skin, 6 rats weighing 250 to 300 g were killed. The following 4 areas of whole rat skin were selected for study; ventral, mid dorsal, right foreleg and left hindleg. Ratio of hexosamine and hydroxyproline to nitrogen and of hexosamine to hydroxyproline for each area of skin was determined in each animal, and mean ratios and

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TABLE I. Mean Chemical Ratios of Various Skin Areas.

Ratio	Skin locations: Right foreleg	Left hindleg	Ventral	Dorsal
Hexosamine (μmole) / Nitrogen (mmole)	3.1 ± .9	2.7 ± .2	2.8 ± .7	2.8 ± .6
Hydroxyproline (μmole) / Nitrogen (mmole)	.26 ±52	119 ±18	107 ±19	124 ±28
Hexosamine / Hydroxyproline	.024 ± .005	.024 ± .004	.026 ± .005	.023 ± .003

TABLE II. Some Chemical Effects of Inflammation.

Days after injury	Hexosamine in: Injured skin	Hexosamine in: Control skin	Hydroxyproline in: Injured skin	Hydroxyproline in: Control skin	Hydroxyproline in: Blood
0	3.8 ± .3	3.8 ± .3	125 ± 13	125 ± 13	2.5 ± .5
3	*10.1 ± 1.7	4.7 ± 1.2	*75.3 ± 4.7	*90 ± 10.7	*8.9 ± .5
4	*14.3 ± 3.3	4.5 ± 1.2	*65.9 ± 53	*88.6 ± 12	*5.5 ± .2
7	*12.8 ± 3.6	*5.8 ± .9	*43.0 ± 8.0	109.0 ± 14	*6.1 ± .3

* Significantly different from 0 time (*i.e.*, $p < .02$).

standard deviations calculated.

Results. Table I indicates that there is no statistically significant variation in chemical analysis with skin locality ($p > .1$). Consequently, any area of skin would be representative of the whole tissue.

Nine male rats weighing 200 to 220 g were injected with croton oil. On 3, 4, and 7 days after injection 3 of these animals were killed. Five ml blood, the necrotic skin (primarily dermis with epithelial papillae), and an equivalent area of skin from the uninjured side were removed. Results of the analyses of hydroxyproline and nitrogen in serum and of hexosamine, hydroxyproline and nitrogen in corresponding skin areas are presented in Table II, along with normal values found in uninjured animals.

Hemolysis in blood samples contributed no appreciable color (tyrosine) to the determination of serum hydroxyproline concentration.

The results were analyzed for statistical significance by the "t" test, and significant changes from normal were found in 12 of the 15 determinations reported in Table II. The necrotic area showed a marked fall in hydroxyproline and a profound increase in hexosamine concentration, while the control area indicated smaller, but parallel, changes in these components. The hydroxyproline to nitrogen ratio of blood demonstrated a 4-fold increase during injury.

The hexosamine-hydroxyproline ratio of the necrotic tissue increased markedly with time after injury, while there was a similar, but considerably smaller change in this ratio for uninjured areas of skin (Fig. 1).

Discussion. One of the major difficulties in studying the chemistry of the inflammatory response is to provide a constant amount of necrotic tissue. At the suggestion of R. H. Pearce, we found that croton oil produced a very reproducible lesion. Intensity of inflammation at any time after injection may be

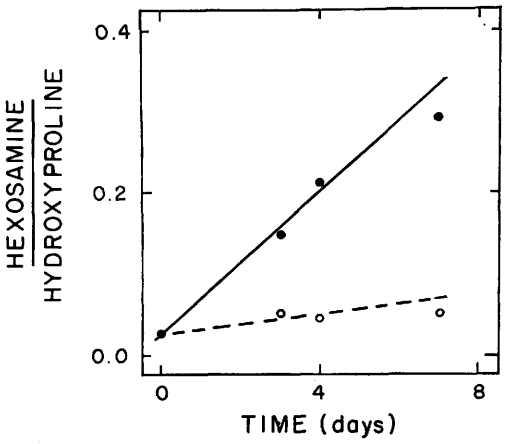

FIG. 1. Change in ratio of hexosamine to hydroxyproline in rat skin with time after injury. Injured tissue (●); uninjured tissue (○).

controlled by strength of irritant used. Pearce found that the effectiveness of various preparations of croton oil differed enormously (personal communication).

Of considerable importance to the study of inflammation is the assurance that variations in skin locality will not produce significant alterations in their chemical analyses. Table I provides this assurance. Consequently, the possibility that the area of skin opposite the site of injury differed in chemical content from area of injection is excluded.

Immediately after injection, the wounded animals lost weight, but after a few days this loss was essentially recovered. Corrections in skin analysis for weight loss or gain could be made from data recently published by us (2), although during the short time used in this study (7 days) alterations in body weight were not significant. This is not true for immature animals weighing 100-150 g (R. H. Pearce, unpublished). The increase in hexosamine and decrease in hydroxyproline or collagen measured during necrosis, was the exact inverse of that reported by Dunphy and Udupa(1) for wound healing. These workers studied preparations containing minimal necrotic tissue, while our animals displayed minimal healing. Presumably, superimposition of both groups of data would provide an overall picture of skin chemistry of inflammation and repair.

An increase in circulating concentration of hydroxyproline is logically consistent with rapid disappearance of this amino acid from skin. Although tyrosine from hemoglobin does not contribute appreciably to hydroxyproline analysis, serum proteins rich in tyrosine undoubtedly do. Concentrations of hydroxyproline in blood reported by Kivirrikko, Liesmaa and Lukainen(3) were considerably smaller than that measured by us. The difference is probably due to the effect of tyrosine upon our analysis. The magnitude of change in blood "hydroxyproline" with inflammation precludes the possibility that all changes in chromagenic material were due to alterations in serum protein tyrosine alone, particularly when the Martin and Axelrod procedure was used to determine hydroxyproline(4).

The striking, almost linear increase in hexosamine to hydroxyproline ratio with time after injury is paralleled by a small change in this ratio in uninjured tissue samples. This latter change is of borderline significance, despite the extremely significant change of each separate component. This is probably due to the fact that when the hexosamine change was significant, the hydroxyproline change was not, and *vice versa* (Table II).

Summary and conclusion. Chemical analysis of rat skin was independent of the area from which the sample was taken. Increases in hexosamine and decreases in hydroxyproline concentration found in skin necrotized with croton oil was paralleled to a lesser degree in uninjured skin. This systemic response was also found as an increase in circulating concentration of hydroxyproline in blood. Despite the statistical significance of these changes, the markedly increasing ratio of hexosamine to hydroxyproline in necrotic tissue with time was not paralleled significantly by the control.

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