duced level of hydroxyproline does not, however, define the identity of the elastin-staining material, since either an increase of true elastin or a degradation of collagen could result in the lower value.

The excess of glucosamine over galactosamine combined with the hyaluronidase lability of the acid mucopolysaccharide staining in sun-damaged skin(9) suggest hyaluronic acid is the major mucopolysaccharide in sun-damaged skin.

Summary. 1. The upper dermis of chronically sun-damaged human skin contains more hexosamine and less hydroxyproline than the upper dermis from unexposed areas. 2. The increased hexosamine confirms the presence of increased acid mucopolysaccharides seen histologically in sun-damaged skin, but the decreased hydroxyproline does not identify the elastin staining material as either elastin or "degraded" collagen. 3. The upper dermis of sun-damaged skin has higher hexosamine and lower hydroxyproline levels than the lower-most dermis of the same specimen, thus the localization of the biochemical alteration corresponds with the localization of the histologic abnormality.

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Is There a Heme-Stimulating Factor in Human Plasma?* (26989)

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Many recent observations have strengthened the original studies of Carnot and Deflandre(1) in experimental animals, suggesting a humoral mechanism controlling erythrocyte production. Plasmas from patients with various hematological disorders have also been found to stimulate erythropoiesis in experimental animals(2). Linman *et al*(3) have suggested that there are probably 2 factors in plasma which exert control over erythropoiesis; one, a thermostable factor which stimulates division of erythroblasts and another factor which is only relatively thermostable, which enhances hemoglobin synthesis.

Various attempts have been made to demonstrate the hemoglobin stimulating factor *in vitro*. Normal rabbit serum has been shown to have a marked stimulating effect on rate of incorporation of C^{14}-labelled glycine into heme by rabbit bone marrow(4). This heme-stimulating factor in serum was thermolabile. There are however conflicting reports regarding the effect of plasma on hemoglobin formation as measured by uptake of radioiron into heme. Using an hemolysate of chicken red cells as the enzymic source and glycine as substrate, Goldberg *et al*(5) failed to show any difference in uptake of radioiron into heme between deproteinized plasmas of normal chickens and of chickens made anemic by

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either bleeding or phenylhydrazine. Uptake of radioiron into heme was much greater when saline was substituted for any of these plasma extracts. In these experiments the iron content of each protein-free extract was determined and sufficient iron had been added to keep constant the total iron content of every extract. Nakao *et al*(6), using an hemolysate of chicken red cells as the enzymic source and δ amino levulic acid as substrate, observed that addition of plasmas from patients with polycythemia, acute myeloid leukemia and aplastic anemia produced an increased incorporation of radioiron into heme when compared to normal human plasma. The plasma from the patient with aplastic anemia apparently stimulated uptake of radioiron into heme a hundred-fold. They consequently postulated that the plasma in these states had heme-stimulating activity. It should be noted that in the latter experiments a protein-free extract was not used except for 2 plasmas in which no heme-stimulating activity was obtained. Although the above authors measured the iron content of each plasma sample, they did not measure the residual iron-binding capacity.

The discrepancy between the results of Goldberg *et al*(5) and Nakao *et al*(6) is of interest since the method of measuring heme formation was similar. The following study was undertaken to determine the cause of these differences. Two obvious factors were the varying size of the iron pools and the varying iron-binding capacities of the plasmas used in the studies of Nakao *et al*(6). The effect of these factors on iron-incorporation into heme was assessed by (1) addition of human plasmas of known residual iron binding capacity, obtained in a variety of hematological disorders and (2) by addition of increasing amounts of iron to a standard aliquot of plasma which thus modified the residual iron-binding capacity as well as the total iron pool of the plasma.

Method. Five ml of chicken red cell hemolysate prepared by the method of Dresel and Falk(7), δ amino levulic acid (4.1×10^{-4}M) and 1 μc radioiron ($Fe^{59}Cl_3$) (specific activity about 2 μc/μg) were incubated with 2 ml of human plasma separated from heparinised blood. The pH was maintained at 7.4 by addition of 0.45 M Tris buffer (2-amino-2-hydroxymethylpropane 1:3 diol). The mixture was incubated with constant shaking at 37°C for 3 hours and the reaction stopped by addition of 1 ml M sodium cyanide. Carrier hemoglobin prepared by the method of Goldberg *et al*(5) was then added to facilitate the isolation of heme. The hemolysate was homogenised in a Waring blender for 3 minutes and centrifuged at 1600 g for 20 minutes to remove tissue debris. The heme was then isolated from the supernatant fluid by the method of Fischer(8) and purified and recrystallised by the method of Shemin *et al*(9). Radioactivity of weighed samples of the isolated heme was determined in a well-type gamma ray scintillation counter with a counting efficiency for iron of 22%. The total number of counts present in the heme was obtained by multiplying the counts per milligram of heme by the total number of milligrams of heme present in the original hemolysate. Results are expressed as percentage of the added radioiron which was incorporated into heme. There is some variation in the iron-incorporating activity of different chicken hemolysates(5). For this reason 2 ml saline was substituted in every experiment for 2 ml of plasma in control flasks and the iron-incorporation into heme with each plasma sample was related to these saline controls in the following way:-

$$\text{Calculated iron-incorporation with plasme sample} = \text{Actual iron-incorporation with plasma} \times \frac{\text{Maximum iron-incorporation of all saline controls}}{\text{Iron-incorporation with respective saline control.}}$$

Serum iron concentration and total iron-binding capacity of plasma were measured by the method described by Ramsay(10).

Results. Comparison of effects of different plasmas. The iron-incorporation into heme was measured when 2 ml samples of the plasmas of 16 patients were added to the system (Table 1). The plasmas were obtained from 5 hematologically normal subjects (Cases 4, 8, 9, 10, 11), 2 patients with a low plasma iron level (Cases 6, 7), one with hemochromatosis (Case 16), one with an un-

TABLE I. Effect of Plasmas of Varying Residual Iron-binding Capacities on Percentage Uptake of Radioiron into Heme.

Case no.	Diagnosis	Hemoglobin (G %)	Serum iron (μg%)	Total iron-binding capacity (μg%)	Residual iron-binding capacity (μg%)	Calculated % uptake of Fe^{59}
1.	Lead Poisoning	9.9	65	489	424	.28
2.	Secondary Polycythemia	16.4	10	336	326	.03
3.	*Idem.*	18.4	60	378	318	.02
4.	Myocardial Infarction	15	72	384	312	.26
5.	Idiopathic acquired hemolytic anemia	10.6	70	308	238	.04
6.	Mild Staphylococcal Infection	14.8	36	270	234	.07
7.	Essential Hypertension	15.7	20	254	234	.31
8.	Hematologically normal	14.8	98	326	228	.10
9.	Myocardial Infarction	13.9	78	300	222	.03
10.	Duodenal Ulcer	14.2	76	274	198	.08
11.	Cerebral Ischemia	15.7	60	222	162	.57
12.	Hypoplastic Anemia	8.3	130	288	158	.62
13.	Monocytic Leukemia	8.4	157	237	80	.045
14.	Aplastic Anemia	5.9	208	276	68	3.15
15.	Myocardial Ischemia	15.4	176	232	56	1.25
16.	Hemochromatosis	14.8	160	198	38	2.08

MAXIMUM UPTAKE OF RADIOIRON OF ALL SALINE CONTROLS = 12%

explained high serum iron (Case 15), and 7 with various disorders involving the hemopoietic system (Cases 1, 2, 3, 5, 12, 13, 14). There was a marked increase of iron-incorporation with addition of the plasmas from 3 patients in each of whom the level of plasma iron was markedly raised and the residual iron binding capacity correspondingly reduced. (Table 1, Cases 14, 15, 16).

Effect of addition of varying amounts of iron to normal plasma. Increasing amounts of iron (4-8 μg,) as ferric ammonium sulfate were added to 2 ml aliquots of a normal plasma so that there was a progressive increase in saturation of the iron-binding capacity(11). There was a sharp increase in iron-incorporation into heme as the iron-binding capacity approached saturation (Fig. 1). The addition of iron in excess of total iron-binding capacity of plasma did not, in the iron concentrations used, alter the percentage

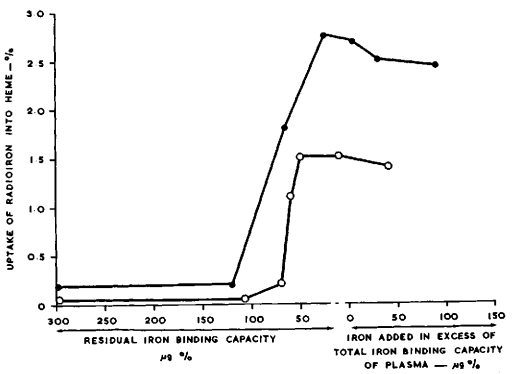

FIG. 1. Effect on heme synthesis of normal plasma to which varying amounts of iron have been added

uptake of radioiron into heme as compared with saturated plasma. Under all circumstances the percentage uptake of radioiron into heme was less in the presence of plasma than in the presence of an equal volume of saline.

Discussion. There is ample evidence for the presence in human plasma of a humoral factor stimulating erythropoiesis. The evidence for a heme-stimulating factor is still unconvincing and every report relating to this should be critically appraised. Nakao *et al*(6) claimed that plasmas from a variety of hematological disorders had heme-stimulating activity. We have used an identical technique to assess this activity in the plasmas of 16 subjects but have found that increased heme-formation occurred with only 3 of these plasmas, in each of which the residual iron-binding capacity was greatly diminished, 68 μg per cent or less. In contrast to Nakao's results the plasmas from patients with secondary polycythemia (cases 2 and 3) and hypoplastic anemia (case 12) showed no evidence of increased heme formation but each of these patients had a residual iron-binding capacity greater than 158 μg per cent. In the plasma of one patient with monocytic leukemia (case 13) in which the residual iron binding capacity was 80 μg per cent, no increase in heme formation occurred.

These results suggested the importance of the residual iron-binding capacity of the plasma samples in a system using radioiron as a tracer for measuring heme synthesis. The so-called heme-stimulating activity of the plasmas observed by Nakao *et al.* could be explained by variability in residual iron-binding capacity. This suggestion is corroborated by the results noted in Fig. 1. An increased iron-incorporation into heme occurred only on addition of plasmas in which the residual iron-binding capacity had been partially or completely saturated by non-radioactive iron. In these experiments one μc radioiron (containing 0.6 μg iron) was added to each flask. Thus a 2 ml sample of plasma with a residual iron-binding capacity of 30 μg per cent could render this quantity of radioiron unavailable for heme synthesis. Lochhead and Goldberg(12), have already shown that radioiron, when added to plasma, is bound to siderophilin and is not readily released for heme synthesis unless certain reducing substances are added. The differences noted in the plasma samples with a residual iron-binding capacity greater than 30 μg per cent may well be accounted for by a varying level of physiological reducing substances present in each tissue system. These considerations have led us to conclude that the apparent heme-stimulating activity of the human plasmas noted by Nakao *et al.*(6) can be explained by the varying residual iron-binding capacity of these plasmas.

Summary. (1) Nakao *et al.*(6) have suggested that plasma samples from a variety of hematological disorders possess heme-stimulating activity as compared to normal plasma. This activity was determined by an *in vitro* technique in which radioiron was used to measure heme synthesis. (2) The present studies have shown that any marked increase in heme formation, determined by the same technique, could be explained by diminution of the residual iron-binding capacity of the plasma sample. (3) In further experiments the residual iron-binding capacity of normal human plasma was modified by addition of varying amounts of non-radioactive iron. Heme formation in the presence of the plasma was directly related to degree of saturation of the residual iron-binding capacity. (4) It is concluded that no definite evidence has yet been obtained for the presence of a specific heme-stimulating factor in human plasma.

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Renal Localization and Persistence of Type 12 Streptococcal M-Protein.* (26990)

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The hypothesis that acute glomerulonephritis results from a hypersensitivity reaction has prompted investigations to explain why such reactions primarily involve the kidney. With respect to the possibility that the antigen might be a foreign protein, the association between Group A streptococcal infection, especially of certain types, *e.g.* Type 12, and the subsequent development of glomerulonephritis has focused attention on streptococcal substances.

Schmidt(1,2) reported that the Group A carbohydrate could be detected, using immunofluorescent techniques, in renal tubular cells of mice for up to 30 minutes following intravenous injection, and that a streptococcal protein could be demonstrated in cells of the reticulo-endothelial cells (RES) for as long as 60 hours. Kaplan(3) has extended the observations on the distribution of streptococcal proteins. He found that when small amounts of acid extracted M-protein were injected into mice, localization occurred largely in the phagocytic cells of the RES and within glomeruli. The M-protein was found to persist in glomeruli for at least 8 days, whereas outside the kidney virtually none was demonstrable by the fourth day.

On the basis of these observations, it might be postulated that the initial event in the pathogenesis of acute glomerulonephritis involves the deposition of M-protein within glomeruli and that subsequently, as circulating antibody against M-protein is produced, this antibody combines with the protein persisting in glomeruli, thereby damaging these structures. However, Kaplan detected no differences in the glomerular localization or persistence of M-protein from Types 1, 5, 12 and 19 Group A streptococci, so that even if the mechanism postulated above is true, the nephritogenic action of certain streptococcal types could not be explained simply on the basis of certain M-proteins having the special property of localizing and persisting in glomeruli.

Furthermore, the possibility exists that the distribution of M-protein observed by Kaplan reflects alterations in the protein produced during the process of its isolation. Thorbecke, Maurer and Benacerraf(4) showed that certain acid treated serum proteins may be rapidly cleared from the circulation by the kidney, whereas the unmodified protein is not.

The present investigation was undertaken to determine whether M-protein isolated with the use of a phage lysin would exhibit the same *in vivo* distribution as the material prepared by the relatively drastic procedure of hot acid extraction. The behavior of both preparations of M-protein was contrasted with that of acid denatured bovine serum albumin (BSA). It was found that phage prepared M-protein did localize and persist in glomeruli of mice, where it was demonstrated for as long as 16 days after a small intravenous injection.

Materials and methods. Animals: Swiss-Webster albino mice of both sexes, weighing 25-30 g were used.

Streptococci: Nass strain, Group A Type 12 streptococci, isolated during a focal outbreak of glomerulonephritis was generously

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