Studies on Serum Haptoglobin in Experimental Connective Tissue Disorders.*[†] (26667)

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Haptoglobin (Hp)[¶] is a serum glycoprotein with the mobility of an alpha-2 globulin characterized by its property of combining irreversibly with hemoglobin(1,2). High serum haptoglobin levels have been found in human subjects in many different pathologic conditions(3,4). Hp values are particularly high in diseases with connective tissue involvement, e.g., rheumatic disease, systemic lupus erythematosus, metastatic cancer. Study of serum Hp in experimental animals and experimental production of hyperhaptoglobinemia are necessary steps to investigate the mechanism of normal and pathological Hp production. The purpose of the present report is to show that different experimentally produced connective tissue disorders in guinea pigs and rabbits are accompanied by very high serum Hp levels. It has been shown that high serum Hp levels occur during development of carrageenan granuloma in rats and guinea pigs(5) and following turpentine injection(6). These findings are now confirmed and extended.

Methods and materials. Guinea pigs were used for induction of carrageenan granulomas(5) and acute scurvy. Rabbits were used for amyloidosis production by biweekly subcutaneous turpentine injections, and for the study of Hp in sera of animals after a single intravenous injection of different enzymes. Enzymes used and their dosage were: Papain (Nutritional Biochemicals, 14 mg/ kg); bovine testicular hyaluronidase (Ar-

mour, 3500 USP units-110 mg/kg); trypsin (Armour, cryst., 3530 units-1 mg/kg); chymotrypsin (Armour, cryst., 14000 Armour units-12 mg/kg); elastase (prepared by a modified Banga method(7) from porcine pancreatin, Armour, having a specific activity of 11.4 azoelastase units/mg protein(8), injected at a concentration of 18 mg protein/ ml, 1 to 3 ml). Control animals received the same volume of sterile saline, or 1.5 mg of ovalbumin in 1.5 ml. In the scurvy experiments two types of controls were used: animals fed the scorbutogenic diet ad libitum, and pair-fed animals, both groups receiving a 2.5 mg supplement of ascorbic acid daily by mouth. The experimental animals received the scorbutogenic diet without Vit. C supplement. Serum samples were stored frozen until used for determinations. Hp was determined by a colorimetric modification of Jayle's "activation" method(9) using ethylperoxide, 0.05 N., as the substrate and guaiacol, Merck, dissolved to 5 ml/l in 0.1M acetate buffer at pH 4.2 as the hydrogen Ten and 20 μ l of each serum were donor. mixed with 0.1 ml of beef cyanmethemoglobin, recrystallized once according to Drabkin(10), in a solution of 0.05 g/l. The volume was made up to 0.2 ml with saline, 5 ml of the guaiacol solution were added and finally 1 ml of the peroxide solution. The color developing in exactly 6 minutes at 30°C was read in a Coleman Jr. Spectrophotometer at 470 m μ in a 13 \times 13 mm rectangular cuvette. A saturation curve, prepared with high titer guinea pig and rabbit serums, was used to establish the optical density corresponding to the peroxidase activity of the saturated hemoglobin-haptoglobin complex. Using this value, the 6-minute optical density tracings were converted to hemoglobin binding capacity (HBC) and expressed in mg hemoglobin bound to 100 ml of serum.

Results. Table I summarizes results of the Hp determinations. Average values were

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Animal	No.	Treatment	HBC, mg % serum	t*
Guinea pigs	16	Normal controls	10.7 ± 2.3	
1.0	7	Pair-fed	27.4 ± 5.9	2.95
	19	Carrageenan granuloma	38.1 ± 6.1	4.5
	14	Acute scurvy	82.6 ± 12.8	5.9
	7	Seurvy and carrageenan	53.0 ± 7.0	5.64
Rabbits	58	Normal controls	70.0 ± 3.8	
	10	Turpentine inj. ; after 3 mo	182.7 ± 15.8	5.0
	5	Same, after 8 mo	118.0 ± 39.5	1.1
	11	Papain I.V.	165.3 ± 13.5	4.7
	5	Elastase I.V.	236.0 ± 76.0	2.17
	9	Hyaluronidase 1.V.	139.0 ± 17.4	3.9
	3	Trypsin I.V.	98.1 ± 10.0	1.5
	3	Chymotrypsin I.V.	53.0 ± 13.0	0.9
	3	Ovalbumin I.V.	100.0 ± 8.6	1.7
	4	Saline I.V.	114.2 ± 21.5	1.6

TABLE I. Serum Haptoglobin Values (Expressed as Hemoglobin Binding Capacity, HBC) of Guinea Pigs and Rabbits Subjected to Various Treatments.

* 95% significance if over 1.9 and 99% significance if over 2.6.

calculated as follows: For guinea pigs the determinations were done for normal controls and for pair-fed animals at the same time as for animals maintained on a vitamin-free diet. The acute scurvy group was bled 3 weeks after the scorbutogenic regimen was started; the carrageenan injected animals were bled on the 8th day after injection which corresponded to the 21st day of the different dietary regimens. Hp values obtained on these samples were then respectively averaged (4th column of Table I).

In the rabbit experiments, blood was taken before treatment and at different intervals after injection. Normal control values were averaged without respect to the time at which blood was taken. For the turpentine treated group the 3-month and 7-month values are given: at 3 months no amyloid was detectable, while at 7 months (and generally after the 4th month) all the animals had amyloid deposits in the spleen and less severe involvement of liver, kidneys and other Animals receiving intravenous entissues. zyme, protein or saline injections were bled before injection and at 9, 18, 50 and 96 hours after the injections. The results presented are the average of the highest values of these time curves, without respect to the time when this value was found. These peak values generally appeared between 9 and 50 hours after injection (Fig. 1). The significance of the difference was calculated between the mean Hp value established as indicated and

the mean of the corresponding normal group (11).

The guinea pigs bearing carrageenan granulomas have a nearly 4-fold mean Hp than the normals, a result similar to that obtained previously using Jayle's iodometric Hp determination(5). Acute scurvy is accompanied by very high serum Hp levels, some 8 times the normal average. It is interesting to note the somewhat lower average HBC in scorbutic guinea pigs bearing granulomas as compared with the simple scurvy group. However, this difference is not significant at the 95% level. The mean HBC of the pairfed groups was significantly higher than that of the normal controls. The reason for this



FIG. 1. Serum haptoglobin (HBC) as a function of time after intrav. inj. into rabbits of different enzymes, proteins and saline.

increase is not apparent although the pairfed animals lost some weight during the experiment.

Repeated turpentine injections in the rabbit resulted in generalized amyloidosis. At 3 months after start of injections serum Hp is about 2.5 times above the normal level. then falls off when amyloid begins to be detectable in the spleen. Seven months after start of treatment, when amyloidosis is widespread, Hp is still higher than the normal average but the difference is no longer sig-Intravenous papain injections in nificant. rabbits according to the technic of Thomas (12) results in a spectacular rise of serum Similar results were obtained with in-Hp. travenous elastase and to a somewhat lesser extent with hyaluronidase injections. Eighteen to 50 hours after papain or elastase injections, serum Hp can be as high as 7fold the initial value. Fig. 1 gives the time course of serum Hp level in some typical experiments. Though a rise can sometimes be obtained even after saline or ovalbumin injections, the mean highest values of these groups are not significantly different from the average of the normal control group. The same holds for trypsin and chymotrypsin injections. Following reinjection of papain at 100 hours, HBC levels were again elevated to values comparable with those obtained after the initial injection.

The behavior of acid polysaccharides was investigated in some cases by uronic acid determinations using a modified Tollens method(13). In contradistinction from the effect of papain(14), both hyaluronidase and elastase injections are followed by a decrease of uronic acid in the serum, followed by a return to initial values after 50 hours.

Discussion. A striking relation between degradative changes in connective tissue and high levels of circulating protein bound polysaccharide was indicated by Catchpole(15), Engel(16), and Pirani and Catchpole(17). Jayle and Boussier(1) adapted this idea to the relation between Hp production and connective tissue degradation and claimed a mesenchymal origin for this glycoprotein. Though there seems to be no quantitative difficulty for this hypothesis(18) a direct proof is not yet available to sustain it.

The present results show that many different treatments produce a considerable rise of serum Hp levels. Some of the treatments used (scurvy, carrageenan granuloma, turpentine and enzyme injections) are of such a nature that one could reasonably suppose a more or less extensive alteration of connective tissue. This has been demonstrated for scurvy(17) and papain(12,14). The carrageenan granuloma and the turpentine abscess both produce rapidly anabolizing and catabolizing new connective tissue. These results agree with clinical experience(3) that rise of serum Hp in humans may be part of a nonspecific inflammatory reaction. However, diet restriction as in pair-fed animals, where none of the above effects would be expected, also produces a significant rise of the serum Hp. It is interesting to notice that the highest Hp levels found during turpentine injections are attained well before the actual appearance of amyloid. With the deposition of amyloid, Hp levels tend to decrease.

Simultaneous determination of uronic acid and of Hp show that there is no parallelism between serum levels of acid mucopolysaccharides and Hp following intravenous injection of enzymes. Papain was the only enzyme producing a rise of uronic acid-containing polysaccharides. Here too, the rise of these mucopolysaccharides appeared well before the rise of Hp, the former reaching their highest level between 10 and 20 hours after injection, the latter at 30 to 50 hours.

The mechanism by which elastase and hyaluronidase lowered serum uronic acid concentration is not evident, though elastase might attack the euglobulin fraction which contains the mucopolysaccharide of serum (19).

Intravenous papain and elastase injections might be used with advantage for an experimental study of the mechanism of biosynthesis and release of Hp.

Summary. Serum haptoglobin was studied in guinea pigs and rabbits in normal animals and after experimental production of connective tissue damage such as in acute scurvy, carrageenan granuloma, turpentine-induced amyloidosis, and intravenous injection of different enzymes (papain, elastase, hyaluronidase, trypsin, chymotrypsin); controls received intravenous ovalbumin and saline. Significant increase of serum haptoglobin was found in acute scurvy (average 8 times normal), carrageenan granuloma (4 times normal) or both combined (5 times normal). and after intravenous injection of papain. elastase and of hyaluronidase (average of 2 to 2.5 times normal). Particularly striking results were obtained with papain and elastase which in some cases produced an increase in haptoglobin up to 7 times the starting value. These methods may be used to obtain reproducible experimental hyperhaptoglobinemia.

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Interrupted Enterohepatic Circulation of Bile and Its Effect on Urinary and Biliary Excretion of I¹³¹.* (26668)

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Any alteration of hepatic physiology markedly affects iodine metabolism. Carbon tetrachloride-induced liver injury results in both increased hepatic retention and decreased thyroid uptake of subcutaneously administered $I^{131}(1)$. Shunting portal circulation by an Eck fistula leads to a decreased thyroid uptake of I^{131} following its intragastric administration(2). Absence of bile in the intestinal tract impairs the absorption of orally administered $I^{131}(3)$. It is not known, however, whether bile loss will modify the metabolism of I^{131} intravenously administered.

The purpose of these experiments was to study the effect of interrupted and uninterrupted enterohepatic circulation on the biliary and urinary excretion of iodide administered intravenously. Also, efforts were made to ascertain the conditions necessary to maintain normal urinary iodide excretion after surgery upon the biliary tract and alteration of normal pathways of bile flow.

Materials and methods. Nine groups (Table I) of male Holtzman rats weighing approximately 150 g were used. Surgery was performed under Nembutal anesthesia.

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