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Excretion of a Methemoglobin-forming Substance in Urine.

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In a peculiar instance of methemoglobin formation first differentiated by Stokvis[1] as a clinical entity "enterogenous cyanosis methemoglobinemia" the urine was shown to contain a substance which was able to convert hemoglobin into methemoglobin *in vitro*. During the course of an investigation of the oxidation-reduction potentials of concentrated dialysates of *Streptococcus viridans* cultures[2] we found that these dialysates were able to form methemoglobin if added to blood, while the original culture medium had no such effect. It therefore occurred to us that the urine of patients suffering from sub-acute bacterial endocarditis might also have this property. On adding such a urine to a dilute solution of blood, the blood turned chocolate brown and the typical spectroscopic bands of methemoglobin became visible.

On the supposition that the urinary constituent causing the formation of methemoglobin must be a relatively high oxidant we tried its action on an acidified solution of potassium iodide and found it capable of liberating free iodine; the urine turned yellowish-brown, and, on the addition of starch, a deep purplish blue. This provided a quick method for the qualitative detection of the methemoglobin-forming substance.

These urines had the following properties in common: 1. On addition to diluted blood there was rapid formation of methemoglobin and the typical spectral bands appeared. 2. On addition of di-methyl *p*-phenylenediamine in acetic acid solution there was instantaneous formation of the bright scarlet red color of Würster's Red. 3. On addition of benzidine in acetic acid solution a bright orange color developed. 4. On addition to a solution containing equal volumes of 10% KI and 2N H_2SO_4 the solution turned yellow, and with starch, deep blue. 5. In an atmosphere of nitrogen, concentrates of the urine restored the color to leuco indigo carmine, leuco methylene blue and leuco 2-6-dichlorphenolindophenol. 6. Owing to continued reoxidation of the dichlorphenolindophenol the ascorbic acid content of positive urines could not be determined accurately by the standard titration method. The results obtained were very low. 7. The active substance was steam-distillable and freely diffusible.

All these reactions are reproducible with water-soluble quinones of the same concentration, including 2-methyl-1,4 naphthoquinone, the vitamin K analogue.

Quantitative Determination of the Methemoglobin-forming Substance as Quinone. To 20 cc of urine, 10 cc of 10% KI and 10 cc of 2N H_2SO_4 are added. The solution turns yellowish brown. This is titrated with n/100 sodium thiosulfate and just before the end-point a few drops of freshly prepared soluble starch are added as an indicator. The end point is extremely sharp and is reached when the blue color disappears. The cc thiosulfate $\times$ 2.7 = mg substance as quinone in 100 cc.

Urine showing a quinone titer of 8.5 mg % was added to blood diluted 1:4, to determine the amount of methemoglobin formation by the decrease in oxygen capacity measured by the manometric method of Van Slyke. (Table I.) When the specimen was dialyzed through a collodion membrane and the dialysate concentrated *in vacuo* the quinone equivalent was raised to 29.8 mg % and the amount of methemoglobin formed was markedly increased.

Since these urines oxidize leuco dichlorphenolindophenol the usual titration method of determining ascorbic acid by reducing this

[1] Stokvis, B. J., *Nederl. Tijdschr. Geneesk.*, 1902, **2**, 678.

[2] Fishberg, E., and Baum, H., *J. Biol. Chem.*, 1938, **123**, xxxv.

TABLES I AND II.
Formation of Methemoglobin by Urine Containing 8.5 mg % Quinone Equivalent.

Blood, cc	Urine, cc	Water, cc	Oxygen cap. vol. %	Meth. Increase vol. %	Meth. % increase	Meth. spect.
2	0.0	2.0	19.34	0	0	0
2	0.25	1.75	18.49	0.85	4.4	+—
2	0.50	1.50	17.91	1.42	7.4	++
2	0.75	1.25	17.11	2.23	11.5	++
2	1.0	1.0	16.01	3.33	17.2	+++
2	1.3	0.7	15.02	4.31	22.3	+++
2	1.6	0.4	13.97	5.37	27.8	++++
2	2.0	0.0	12.99	6.34	32.8	>++++
Formation of Methemoglobin by Dialyzed Urine Containing 29.8 mg % Quinone Equivalent.						
2	0.0	2.0	19.93	0	0	0
2	0.4	1.6	15.99	3.93	19.7	+++
2	0.7	1.3	13.96	5.97	29.9	++++
2	1.0	1.0	10.66	9.27	46.5	>++++
2	1.4	0.6	8.84	11.09	56.7	>++++
2	1.7	0.3	7.27	12.66	63.5	>++++
2	2.0	0.0	5.00	14.93	74.9	>++++

TABLE III.
Influence of Methemoglobin-forming Material on the Titration of Ascorbic Acid.

Ascorbic acid present, mg	Dichlorphenol indophenol, cc	Urine added, cc	Quinone by titration, mg	Ascorbic acid found, mg/100 cc	% found of ascorbic acid present
.0176	.73	0	0	17.6	100
.0176	.60	0.2	.018	14.47	82.2
.0176	.535	0.3	.028	12.90	73.3
.0176	**.42**	**0.5**	**.045**	**10.13**	57.5
.0176	.30	0.7	.064	7.24	41.2
.0176	.125	1.0	.091	3.01	17.1
.0176	.071	1.1	.100	1.71	9.7

dye to its leuco form obviously cannot be employed. Methemoglobin-forming urines titrated for ascorbic acid showed a daily output of 1.3, 1.66, 1.1, and 2.1 mg in contrast to non-methemoglobin-forming urines which showed the normal values of 10-25 mg. For a direct demonstration varying quantities of positive methemoglobin-forming urine were added to 0.1 cc of a solution of ascorbic acid containing 17.6 mg % and the mixture was then titrated with dichlorphenolindophenol. As seen from Table III, the concentration of ascorbic acid so measured is an inverse linear function of the amount of positive urine and does not in any way represent the amount of ascorbic acid actually present.

Over 9,000 specimens sent to the routine laboratory from the wards were tested for the presence of the methemoglobin-forming substance. Those which were positive over periods of several days and showed values between 3 and 10 mg % were from cases of sub-acute bacterial endocarditis, rheumatic fever, and acute arthritis. There were negative intervals between the positive periods. In a special group of which we had seven cases the excretion of the methemoglobin-forming substance was continuous and showed extremely high values up to 40 mg %. These included a 69-year-old man admitted for continued epistaxis who had subsisted on a diet completely free of fruits and vegetables for a period of over 40 years, an infant suffering from acute scurvy, and 2 cases of pneumococcus peritonitis in children following nephrosis. The urines of both these cases were continuously negative over a stay of several months in the hospital but turned

positive for the methemoglobin-forming substance about one week before the blood culture became positive for pneumococci. There were also two cases of hemorrhagic colitis.

Summary. A substance capable of forming methemoglobin from blood *in vitro*, and which can be quantitatively estimated as quinone, has been found to be excreted in certain pathological states. This urinary constituent interferes with the titration of ascorbic acid.

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Influence of Pituitary Adrenotrophic Hormone on Lymphoid Tissue Structure in Relation to Serum Proteins.*

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Lymphoid tissue mass[1,2] and blood lymphocytes[3] are under pituitary adrenotrophic hormone influence. Loss in lymphoid tissue weight and the lymphopenia following hormone injection suggested an approach to the problem of the fate of lymphocytes.

This paper presents a preliminary description of lymphoid tissue histology after injections of adrenotrophic hormone into normal mice and rabbits. Histological alterations observed suggested study of effects of adrenotrophic hormone on serum proteins and antibody formation.

Histology. Single, subcutaneous injections of 1 mg adrenotrophic hormone[4] in 0.5 ml of water into mice or 10 mg in 2 ml solution into rabbits produce alterations in lymphoid tissue histology. Within 1 to 3 hours, degenerative changes are evident in lymphocytes in germinal centers of lymph nodes, in Malpighian follicles of the spleen, in the thymic cortex, and in Peyer's patches. Three to 6 hours after injection, depletion of lymphocytes in lymphoid tissue occurs, accompanied by marked edema. Degenerative changes are characterized by pycnosis and nuclear fragmentation in small or medium-sized lymphocytes. Phagocytosis of nuclear debris and red cells is accompanied by polymorphonuclear invasion of lymphoid tissues. Restoration of normal lymphoid structure begins after 9 hours and is still continuing 24 hours after hormone injection. These changes do not occur in adrenalectomized mice injected with adrenotrophic hormone.

The loss of lymphocytes from lymphoid tissue and from the circulation, and their absence from large capillary beds, suggested dissolution of these cells. In this event, significant quantities of protein nitrogen would be released for metabolic use. A portion of the protoplasmic nitrogen of the dissolved lymphocytes was sought in the serum proteins.

Serum Protein Observations. Total serum proteins and the specific gravity of whole blood were determined on tail vein blood by Kagan's methods.[5,6] No significant alterations in whole blood specific gravity occurred. The serum protein data are presented in

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1 Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 132.

2 Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., *Ibid.*, 1943, **54**, 135.

3 Dougherty, T. F., and White, A., *Science*, 1943, **98**, 367.

4 Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

5 Kagan, B. M., *J. Clin. Invest.*, 1938, **17**, 373.

6 Kagan, B. M., *J. Lab. and Clin. Med.*, 1941, **26**, 1681.