Summary. The vital capacity of normal subjects is not diminished by exercise severe enough to cause marked dyspnea. It is concluded that the dyspnea of exertion of normal subjects is not due to impaired pulmonary function consequent to congestion of the lungs.

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The Urinary Pigments in Four Cases of Alcoholic Pellagra.

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Beckh, Ellinger and Spies[1] employed a very simple procedure for detecting and estimating porphyrin in the urine in 20 cases of pellagra. This consisted of extraction of the acidified urine with ether, after which the ether was extracted with a small amount of 25% HCl. The presence of a red or red violet color in this solution was considered due to porphyrin, and was estimated colorimetrically by comparison with a standard porphyrin solution.

In the present investigation the results of the B.E.S. (Beckh-Ellinger-Spies) test have been compared with the values for coproporphyrin obtained by means of a slight modification of the Fikentscher[2] method (estimation of red fluorescence of coproporphyrin in ultraviolet light, with the Zeiss stufenphotometer). Quantitative determinations were made in 3 of 4 cases of typical alcoholic pellagra.* In the first case but a single 24-hour urine sample was available prior to nicotinic acid therapy; this was examined qualitatively by the usual acetic and ether, followed by 5% HCl, extractions. The amount of porphyrin did not appear to be appreciably increased.

In a series of 5 normal individuals the range of coproporphyrin with the quantitative method employed, was 16-62.8γ per day. In the 3 pellagra cases in whom determinations were made, the following results were obtained:

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1 Beckh, W., Ellinger, P., and Spies, T. D., *Quart. J. Med.*, 1937, **30**, 305.

2 Fikentscher, R., *Biochem. Z.*, 1932, **149**, 257.

* Two of these cases were on the medical service of the Johns Hopkins Hospital, Baltimore, and 2 were on the medical service of the Minneapolis General Hospital. The writer is indebted to Drs. Warfield Longcope and George Fahr, respectively, for access to the clinical data and urines of these cases.

Case 2			Case 3			Case 4		
Date	24-hour urine coproporphyrin in γ	B.E.S. Test	Date	24-hour urine coproporphyrin in γ	B.E.S. Test	Date	24-hour urine coproporphyrin in γ	B.E.S. Test
6-12	65.7	+	8-25	241.0	+	9-25	101.1	+
6-13	218.6	—	8-26	283.1	+	9-26	232.3	+
6-14	109.3	—	8-27	358.8	—	9-28	452.4	—
	nicotinic acid begun		8-28	425.0	—		nicotinic acid begun	
			8-29	174.0	—			
6-15	81.6	—	8-30	264.9	—	9-30	537.1	—
6-16	140.0	—	8-31	263.8	—	10- 1	157.0	—
6-18	95.2	—	9- 1	299.2	?	10- 5	93.9	—
6-22	57.8	—		nicotinic acid begun	(faint color)	10-10	129.5	—
6-23	85.4	—						
			9- 4	57.1	—			
			9- 6	99.7	—			
			9- 8	144.9	—			
			9-10	107.4	—			

It is seen that the amounts of coproporphyrin were distinctly increased prior to nicotinic acid therapy, decreasing significantly, although not into the normal range, thereafter. It should be pointed out that the persistently somewhat elevated values may be attributed readily enough to chronic alcoholism alone.[3, 4]

The above data fail to reveal any correlation between the amount of porphyrin as determined fluorimetrically, and the B.E.S. test. It will be noted that the test was positive when the 24-hour coproporphyrin was as little at 65.7γ, and negative when it was elevated to 537γ. Further study of urine samples exhibiting positive B.E.S. tests, revealed that the color was not due to porphyrin but to another red pigment which will be mentioned more fully below. If the ether extract, as obtained in the B.E.S. test, is first extracted with 5% HCl, this solution, although nearly or entirely colorless, will often be seen to exhibit faint porphyrin absorption at 550mμ. If the ether is next extracted with 25% HCl a reddish pink color appears, but spectroscopic examination now fails to reveal any porphyrin absorption. From this it is quite clear that the B.E.S. test is not specific for porphyrins, although there is no doubt that large amounts of porphyrin, if present in the urine, would give rise to a positive test. The Beckh-Ellinger-Spies method is even less satisfactory as a quantitative procedure, since disturbing colors, particularly those due to urobilinogen derivatives, interfere. It was at first thought that mesobiliviolin might be present in sufficient amount to be productive of positive tests, but subsequent study of a number of urines rich in urobilinogen (the mother substance of mesobiliviolin) has shown that this is not the case.

[3] Brugsch, J. T., and Keys, A., *Proc. Staff Mayo Clinic*, **1937, 12, 609.**

[4] Franke, K., *Z. f. klin. Med.*, 1936, **130**, 222.

Urine from the first case showed a positive B.E.S. test in Baltimore when the fresh urine was examined.† This was negative when the test was carried out in Minneapolis. It was noted that the toluene preservative was pinkish red.‡ More red pigment was extracted from the urine after acidification with glacial acetic acid. It was impossible to remove the red pigment from the combined ether-toluene fraction, by shaking with either 25% HCl or 10% NH_4OH. Crystals of the red pigment were readily obtained upon concentration of the ether-toluene solution. The yield was approximately 2 mg. The substance was sparingly soluble in ethyl acetate and was recrystallized from hot ethyl acetate, also from ethyl acetate and a relatively large volume of petroleum ether, in which the pigment is quite insoluble. The crystals were in the form of long needles (Fig. 1). Unfortunately, they sublimed gradually above 150°C, so that a melting point determination was excluded.

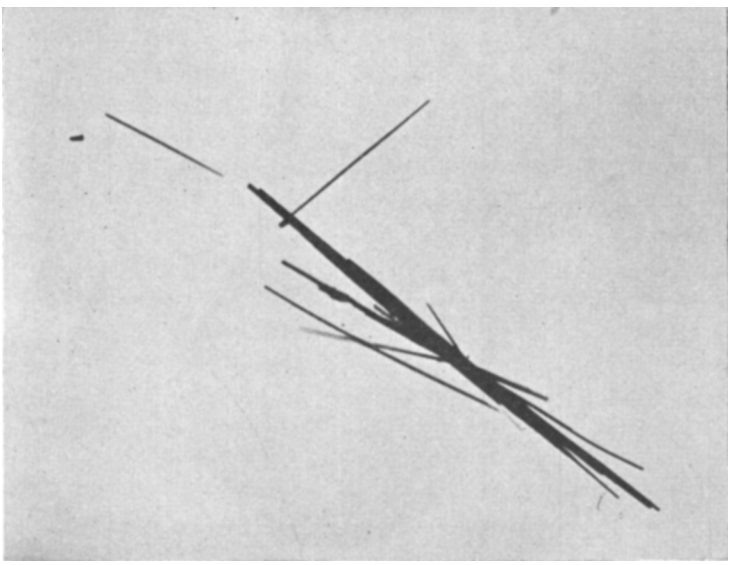

FIG. 1.
Crystals of red pigment from case 1. × 150.

The urine from Case 2 contained a very similar red pigment. Again the toluene preservative was pinkish, although the concentration of substance was not as great as in Case 1. Crystals were not ob-

† The writer is indebted to Dr. W. Halsey Barker for reports of the B.E.S. tests on the fresh urines of cases 1 and 2, and for transmission of these urine samples to Minneapolis.

‡ Spies has observed that the toluene preservatives of pellagra urines, particularly after transportation, are often pink or reddish. Personal communication.

tained. A red pigment was also noted in the urine of Case 3, although in this instance the toluene preservative was not pink. In order to produce conditions similar to those for Cases 1 and 2, where the urines had been transported a long distance under toluene, a 24-hour urine sample from Case 3 was kept under toluene for 10 days, with frequent shaking daily. The toluene, however, did not become colored. The red pigment in Case 3 differed further in that it was extractable from ether by means of 25% HCl, although with difficulty.

In spectroscopic character the red pigments noted in the first 3 cases were quite similar. In each instance there was broad, diffuse absorption, with poorly defined margins, in the region from 5700-5300Å. An ethyl acetate solution of the crystals from Case 1 exhibited absorption from 571-516mμ, with a slight break between 548-536; the redward half of this absorption was somewhat more intense. The absorption of this solution in the ultraviolet was photographed by Dr. Irwin Vigness of the Division of Biophysics in the University Hospital. This revealed well defined, although rather weak absorption at max. 2870Å. Similar absorption (max. 2900Å) was noted in an ethyl acetate extract from Case 3, but was not observed in such an extract from Case 2. This may have been explained simply on the basis of dilution, since the absorption in the most concentrated solution was relatively weak.

It is important to note that the red pigment was not observed in the urines of Cases 1, 2, and 3 after nicotinic acid therapy. This was true of toluene preservative, ether extract, and secondary 25% HCl fraction.

The urine from Case 4 exhibited a positive B.E.S. test in the first 2 days' collection. The ether extracts from 400 cc amounts of these urines, however, failed to show any pink or red color. This was also true of the subsequent 25% HCl extracts from the ether. Because of bad emulsions the process of ether extraction was much slower with the larger amounts and it could only be assumed that the pigment was labile and had faded during the manipulations.

Coproporphyrin III dimethyl ester was isolated from the total 8-day urine collection prior to nicotinic acid therapy in Case 3. The substance crystallized slowly in the rosettes of prisms characteristic of copro-III, and after 4 recrystallizations the melting point was sharp at 164-165°C. The yield was approximately 0.5 mg. The significance of this finding is not clear, but, at least insofar as the one case is concerned, indicates that the disturbance of pigment metabolism was similar to that known to exist in lead and arsenic poisoning.[5]

[5] Watson, C. J., in *Oxford Medicine*, Chap. IX, p. 228.

Red urine has not been described as a symptom of pellagra, either endemic or alcoholic. The amounts of urinary porphyrin reported by Beckh, Ellinger and Spies were in many instances so large that the urine might have been expected to be red, since the amounts were as large as are often encountered in the wine red or dark red urines of idiopathic porphyria. This discrepancy is probably explained by the fact that the simple procedure they used is not specific for porphyrins and is not suitable for quantitative determination of the porphyrins. In the 3 cases discussed here, in which fluorimetric determinations were made, the per diem amount of coproporphyrin in no instance exceeded 0.6 mg (600γ), while Beckh, Ellinger and Spies reported 4 cases in which the amount was *over* 100 mg per liter, 3 in which the amount was between 10-100 mg, and 3 in which it was between 1-10 mg. In all of the present cases red pigments have been encountered differing from porphyrins or known hemoglobin derivatives. To what extent these pigments are related is not known. Considerable similarity in spectroscopic absorption was observed. The possibility must be considered that a common parent substance is altered variously in different urines and under different conditions, possibly undergoing esterification or conjugation. A change of the latter type was suggested particularly in the first of the present 4 cases, in which 25% HCl extracted a red pigment from the ether extract of the fresh urine, but later failed to remove it.

Prof. J. F. McClendon, Department of Physiological Chemistry, University of Minnesota, suggested that these red pigments might be indigo derivatives. Indirubin (indigo red, indipurpurin) exhibits similar absorption in xylene at 561 and 522 mμ (max.) and crystallizes in crimson needles which sublime without melting. The possibility is being investigated that this is the red substance which has been encountered in pellagra urines.

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A Rapid and Sensitive Method for Bioassay of the Adrenal Cortical Hormone.

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In view of the ever growing interest in the physiology of the cortical hormone, it seemed of importance to develop a method