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Coproporphyrinuria (Type III) in Acute Poliomyelitis.*

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The recent epidemic of poliomyelitis in Minneapolis has provided opportunity to investigate the urinary coproporphyrin excretion in this disease. The need for such a study was suggested by a number of facts relating to the normal and pathological physiology of the porphyrins. Chief among these was the observation of Klüver,[1,2] that the white matter of the central nervous system of warm-blooded animals regularly contains a small amount of coproporphyrin. Klüver's study† indicated that this was probably the Type III isomer, at least from the standpoint of its solubility characteristics. Our own studies bearing on this point, to be described in detail elsewhere, have likewise indicated the presence of the Type III isomer, on the basis of the differentiai precipitation or "fluorescence quenching" method.[3]

In addition to the physiological occurrence of coproporphyrin in the central nervous system, the markedly increased urinary excretion of the Type III isomer in lead and arsenic poisoning, and, in association with uroporphyrin, in intermittent acute porphyria (diseases in which the nervous system is commonly affected), constituted additional reasons for investigating the porphyrin metabolism in poliomyelitis. This disease has in fact been confused clinically with acute porphyria,[4] an additional instance of the latter type having come to our attention in recent weeks.

Still another reason for investigating the urinary coproporphyrin in poliomyelitis is the occurrence of unexplained hypertension in this disease, especially in the bulbar type. This has been observed rather frequently in the present epidemic. So far as can be determined, hypertension in poliomyelitis has been mentioned specifically in but one report in literature (Salus[5]) although it has been referred to in association with lesions of the brain stem.[6] It is well known, of course, that hypertension is a relatively common manifestation of intermittent acute porphyria, with or without bulbar manifestations, although the relationship is as yet unexplained. Carrié[7] classifies the hypertension of porphyria, and of lead poisoning, as vasospastic in type, designating it as "porphyrinopathic."

The present material consisted of 64 cases of poliomyelitis studied at various stages of the disease, and representing various types and degrees of severity. One hundred and ninety determinations of total coproporphyrin were made in these cases, on aliquots of 24-hour urine samples, a modification of Fikentscher's method[8] being used.‡ Fifty-one isomer analyses‡ were made by means of the differential precipitation of the esters of coproporphyrin I and III in 30-35% acetone in the cold.[3,9] The results obtained

* Aided by a grant from the John and Mary R. Markle Foundation, New York City, and the National Foundation for Infantile Paralysis, Inc.

1 Klüver, H., *J. Psychol.*, 1944, **17**, 209.

2 Klüver, H., *Science*, 1944, **99**, 482.

† Personal communication.

3 Schwartz, S., Hawkinson, V., and Watson, C. J., *Science*, 1946, **103**, 338.

4 Waldenström, J., *Acta Psychiatrica et Neurologica*, 1939, **14**, 375.

5 Salus, F., *Klin. Wchnschr.*, 1932, **11**, 1542.

6 Page, I. H., and Corcoran, A. C., *Arterial Hypertension. Its Diagnosis and Treatment*, Year Book Publishers, Chicago, 1945, p. 20.

7 Carrié, C., *Die Porphyrine, Ihr Nachweis, Ihre Physiologie und Klinik*, Leipzig, G. Thieme, 1936.

8 Fikentscher, R., *Biochem. Z.*, 1932, **249**, 257.

‡ Details of these procedures will be given in a forthcoming publication.[9] Of the two alternative methods of isomer analysis, A and B, the latter was used in the present study.

9 Schwartz, S., Hawkinson, V., Cohen, S., and Watson, C. J., *J. Biol. Chem.*, in press.

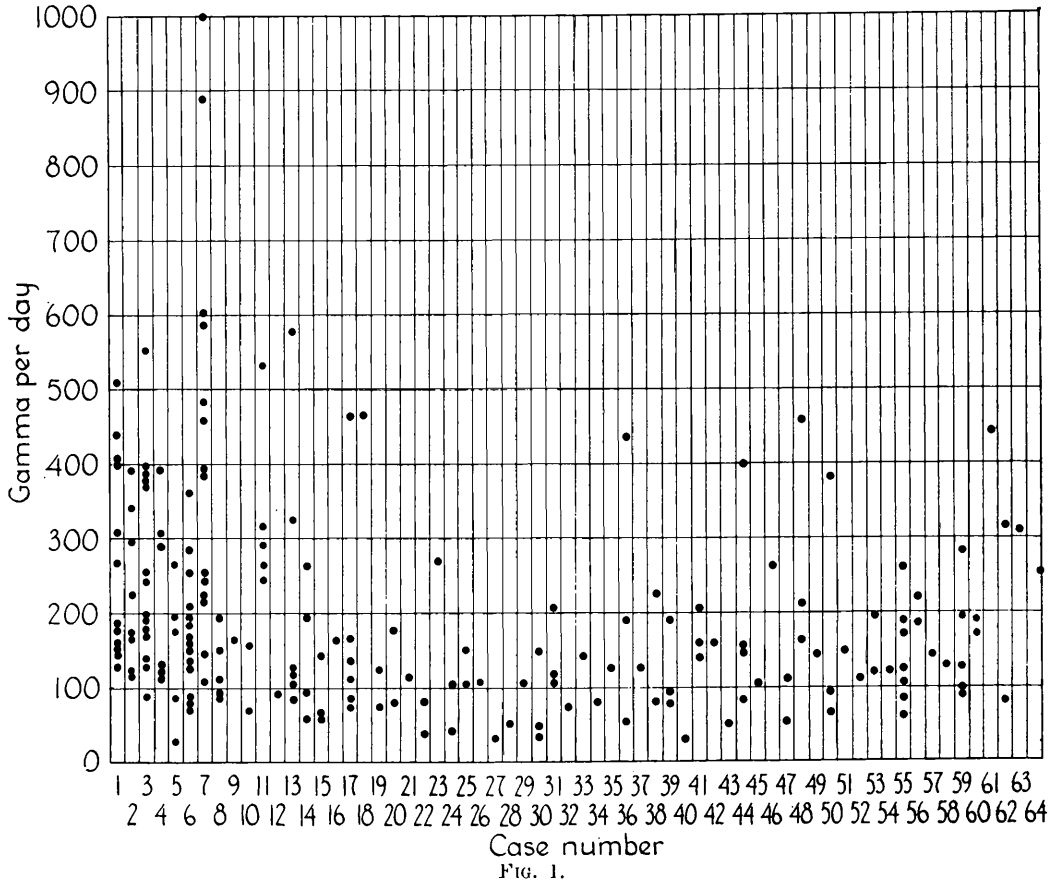

FIG. 1.

Values for total coproporphyrin in γ per 24 hours, as determined 190 times in 64 cases.

have been sufficiently uniform that there can be little doubt about the essential phenomenon which they represent, whatever its significance. The purpose of the present report is to record these observations.

In Fig. 1, the results of the total coproporphyrin determinations are shown as carried out on the 24-hour urine samples. In Fig. 2, 51 isomer analyses as carried out on 38 cases, have been plotted in terms of both total coproporphyrin and percentage of the Type III isomer, as compared with the normal range (the shaded area in the left lower corner of Fig. 2).

Data have been presented,[10] and these will be considered in more detail elsewhere, which reveal that the normal range of urinary coproporphyrin in adult human beings is from 20-100 γ per day, of which 8 to 35% is the Type III isomer, the remainder being Type I. Comparison of these figures with those given above in Fig. 1 and 2 reveal the frequency of increase of the total coproporphyrin and of the proportion of the Type III isomer which characterize the urine in cases of poliomyelitis.

Since the possibility existed that some peculiarity of the urine in these cases might be affecting the differential precipitation technic in such a way that Type I isomer was behaving in part like Type III, it was clearly necessary to control the observations by means of isolation of the crystalline coproporphyrin ester and determination of its melting point. This has been done from the pooled final solutions used for isomer analysis, as obtained from 19 urine samples;

[10] Watson, C. J., Proc. Am. Soc. Clin. Invest., 1946, *J. Clin. Invest.*, in press.

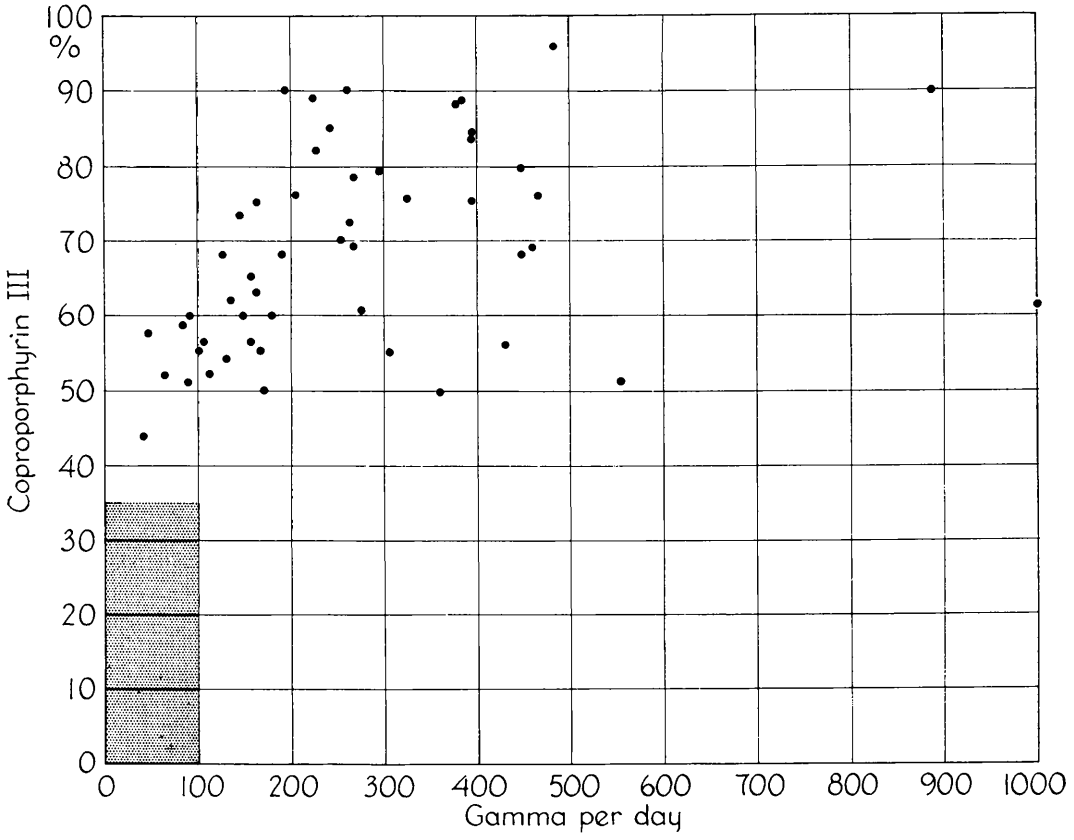

FIG. 2.

Total coproporphyrin (abscissa) and percentage of type III isomer (ordinate) in 38 cases (51 analyses). The shaded area in the lower left corner represents the normal range.

also by direct isolation[11] from five 24-hour urine samples, 2 from Case 7, and 1 each from Cases 1, 2 and 59 (case numbers refer to Fig. 1).

The results of these isolations are given in Table I, in which it is evident that the Type III coproporphyrin isomer is clearly present in excess.

One case, which was not included among the above, deserves special comment. In this instance (L.H., ♂, 32), despite a value of 642 γ for total urinary coproporphyrin, the isomer analysis revealed but 18% of Type III. This patient differed from all the others, however, in having a mild jaundice, and in having been exposed to 2 cases of jaundice among the members of her own family, about one month previously. In addition to jaundice, she exhibited a tender liver for a short period; also, evidence of hepatic functional impairment including urobilinogenuria, positive cephalin-cholesterol flocculation and thymol turbidity tests, and abnormal bromsulfalein retention. While all the latter might possibly be regarded as due to toxic injury of the liver secondary to poliomyelitis, the presence of jaundice, the history of close contact with infectious jaundice, and the tender liver, strongly favored a specific virus hepatitis. Yet there was also evidence suggestive of poliomyelitis. The neck flexion sign was strongly positive, there was muscle weakness, especially in the deltoids and triceps, and the spinal fluid contained 17 cells per cu mm (100% lymphocytes). Although, as noted above, the majority of the increased coproporphyrin was Type I, the actual amount of coproporphyrin III in the 24-hour sample, *i.e.*, 117 γ, is far above the normal. Thus, it is evident that both isomers

[11] Grinstein, M., Schwartz, S., and Watson, C. J., *J. Biol. Chem.*, 1945, **157**, 323.

TABLE I.
Percentage of Type III Isomer, and Melting Points of Crystalline Methyl Esters Isolated from Various Cases in the Series.

Case No.	Total coproporphyrin in γ per 24 hr	% of Type III isomer	Crystal habitus	Melting point (°C) of crystalline methyl ester			Melting point (°C) of Cu complex of methyl ester	
				1st crop	1st recryst.	2nd recryst.	1st crop	Recryst.
1. (H.M.)	441	80	Rosettes of prisms*	130-151	128-135			
2. (I.D.)	390	75	" " "	130-142	130-132		200-210	201-209
59. (W.S.)	378	88	" " "	131-143	130-131			
7. (K.P.)	1000	62	" " "	160-183	152-160	130-132	199-209	200-206
7. (K.P.)	890	90	" " "	130-141	130-134			
45-63†			" " "	150-183	140-142	130-132		

* The tetramethyl ester of coproporphyrin III crystallizes characteristically in rosettes of straight prisms which exhibit dimorphism in melting point: 135°; 144°C; 167-170°C.[14]

† Pooled final solutions used for total coproporphyrin determinations.

‡ Pooled crystals from cases 1, 2, and 59, were converted to the Cu complex which crystallized from $CHCl_3$: CH_3OH after preliminary chromatographic purification.[11] The Cu complex of coproporphyrin III methyl ester melts at 206-207°C (corrected). All of the above melting points are corrected.

were increased, a finding which is fully compatible with the belief that the patient had both diseases simultaneously.

Discussion. The data thus far obtained do not indicate the existence, in poliomyelitis, of a correlation of the degree of increase of the urinary coproporphyrin with severity of the disease, prognosis as to death or recovery, or extent of residual paralysis; or as to type of disease, whether bulbar, spinal respiratory, or mixed. Nevertheless, in the 10 fatal cases which have been studied, all the determinations of the total coproporphyrin and the percentage of Type III isomer were significantly, and as a rule markedly, elevated regardless of the stage of the disease. In a number of instances, high values have been encountered for many days and in some cases for a number of weeks beyond the febrile period, likewise in some cases who have had a relatively mild attack and are now recovering. The majority of the values below 100 γ per 24 hours, as noted in Fig. 1, were obtained during the convalescent period. With reference to Fig. 1, it may also be noted that the levels of coproporphyrin excretion in an individual patient have often shown wide variation.

For example, in Case 1, the first determination made on the 4th day of the disease was 186 γ after which it fell to 125 γ and 154 γ on the 6th and 8th days after onset, then rose to 306 γ on the 9th day only to fall again to approximately 150 γ for 3 days, with a further rise to 400 γ from the 13th to 16th days after onset. On the 19th day the value was 264 γ and this was followed by a rise to the highest level of 505 γ on the 21st day. The last value obtained was 441 γ on the 24th day, death ensuing on the 27th day.

By contrast, in Case 2, the highest levels were obtained on the 3rd through the 9th days of the disease with a progressive fall through the 16th day of the disease to a level of 120 γ, death ensuing on the 19th day after onset.

[14] Fischer, H., and Orth, H., *Die Chemie des Pyrrols*, Bd. II, 1 Hälfte, Akad. Verlagsgesellsch. Leipzig, 1937.

In Case 7, in which the highest levels were noted, the first determination, on the 9th day of the disease, was 216 γ, after which it increased to 392 γ on the 11th, 455 γ on the 13th, 890 γ on the 14th, then fell to an almost normal level of 105 on the 20th day only to rise again to a 1000 γ level on the 23rd day, without any marked change in the clinical condition of the patient. The level then again fell to 146 γ on the 26th day of the disease with exitus on the 30th day after onset.

In Case 3, determinations were made from the 20th to the 44th day of the disease, with levels ranging from 195 γ on the 20th day to a high of 550 γ on the 26th day, with several subsequent rises and falls and finally a decrease to the normal range on the 44th day after onset. This patient went on to a good clinical recovery.

It is thus apparent that the urinary coproporphyrin values have not been of assistance in prognosis nor could we correlate them with the current clinical status of the patient. Insofar as hypertension was concerned, there was also little, if any, correlation. It was true that in nearly all cases with elevated blood pressure in which porphyrin determinations were made, increased amounts were found. The only 2 exceptions were in pregnant women whose acute attacks were 3-4 months in the background and whose hypertension was believed to be on the basis of toxemia of pregnancy. There were many individuals, however, whose blood pressure was normal in spite of markedly increased porphyrin values. If the elevated blood pressure is "porphyrinopathic" it would have to be assumed that constitutional or other factors were responsible for the differences encountered. In one instance the blood pressure and porphyrin values were noted to decrease concomitantly, but in other cases marked fluctuations in blood pressure were recorded without evident correlation with the urinary porphyrin values.

Fever[12,13] and epidemic hepatitis,[10-13] as well as ordinary hepatic functional disturbances, whether parenchymal or mechanical in type,[10-13] have been shown to be associated with an increase of the Type I coproporphyrin isomer in the urine. Until now, infectious disease has not been found to cause an increased excretion of coproporphyrin III. It is of much interest in this connection that the increase in cases of infectious hepatitis is regularly due to the Type I isomer,[10] yet the virus of this disease is similar in some respects to the virus of poliomyelitis, both being relatively resistant and both being found in the gastrointestinal tract. Thus, it would appear logical to seek for an explanation of the difference in porphyrin excretion in the 2 diseases in the effect on the organism of the neurotropic as versus the hepatotropic nature of the respective viruses. The possibility must be considered that the excessive coproporphyrin III excretion observed in the present cases of poliomyelitis is in some way related to a disturbance of the coproporphyrin of the central nervous system. Another possibility is that it is derived from myohemoglobin, as a result of the disturbance in muscle physiology. This appears unlikely in view of the fact that marked increases have been noted in cases of relatively pure bulbar type with little or no muscle paralysis, also in early cases prior to any demonstrable muscle weakness.

Inspection of the isomer data shown in Fig. 2 reveals that while coproporphyrin III is clearly preponderant there is also, in many instances, a distinct increase of the Type I isomer. The simultaneous occurrence of marked urobilinogenuria in a number of these cases suggests that the increase in coproporphyrin I may be due to hepatic functional impairment.

Further studies are in progress with respect to the significance of these observations. The increased excretion of coproporphyrin III establishes a biochemical difference between 2 specific virus diseases, *i.e.*, poliomyelitis and infectious hepatitis. It is desirable to ascertain whether other neurotropic virus affections are similarly characterized, and whether virus diseases of dissimilar type, such as atypical pneumonia or psittacosis, if they exhibit any increases in

[12] Watson, C. J., *J. Clin. Invest.*, 1936, **15**, 327.
[13] Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

urinary porphyrin at all, are, like infectious hepatitis, characterized by an increase of the Type I isomer.

Summary and Conclusion. A considerable excess in the excretion of coproporphyrin III has been commonly noted in a series of urine samples from cases of acute poliomyelitis. The total urinary coproporphyrin is usually in the range of 100-500 γ per 24-hour sample, with from 50-90% of Type III isomer, as compared with 20-100 γ and 8-35%, normally. This abnormal excretion is contrasted with the marked increase of Type I isomer previously found to characterize the urine in cases of infectious hepatitis.

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Experimental Evidence of the Secretion of Urine by the Fetal Kidney.*

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In the fetal rat, ligation of the ureter causes hydronephrosis and ligation of the urogenital papilla causes filling of the bladder.[1] The rate of formation of fluid by the fetal kidney is surprisingly rapid and it may be accelerated experimentally by ligating the renal pedicles of the mother or by injecting a highly concentrated solution of urea under the skin of the fetus.[2]

The present study which grew out of the experiments noted above, has been designed to determine whether this fluid is actually urine or merely a transudate of the fetal blood. In order to attain this objective, we have studied the blood and contents of the bladder of the fetus and also those of the mother.

The material for analysis was collected in the Department of Anatomy from rats in which the pregnancies were dated from the moment of observation of coitus. On the 21st day of pregnancy (20 days and 17 hours ± 2 hours), the mother was anesthetized with ether and her abdomen was opened surgically. Each fetus of a litter was transferred to the abdominal cavity of the mother[3] and its urogenital papilla was ligated by means of an unraveled strand of surgical silk. Then the abdominal incision of the mother was closed by suturing and the anesthesia was discontinued. On the 22nd day of pregnancy and about 2 hours before autopsy, the urinary papilla of the mother was ligated by means of braided silk (Champion No. 6). A few hours before the estimated time for normal parturition, the mother and her fetuses were killed by decapitation (killed at about 21½ days after coitus). Blood emerging during decapitation was allowed to drop into small vials which previously had been moistened with heparin solution. The maternal urine was secured by puncturing the bladder with a hypodermic needle (gauge 27) attached to a 10 cc syringe. The fetal urine was obtained by puncturing the bladder with a micro-pipette prepared from glass tubing and equipped with a small rubber bulb (a "policeman," commonly used in chemical laboratories).† By pooling the samples from the fetuses of one mother or, more rarely, from those of several mothers, it was possible to obtain sufficient material for the analyses.

The chemical methods employed were micro-modifications of the following standard

* Aided by grants from the John and Mary R. Markle Foundation and from the medical research funds of the Graduate School.

1 Wells, L. J., *Anat. Rec.*, 1946, **94**, 504.

2 Wells, L. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 287.

3 Wells, L. J., *Anat. Rec.*, 1946, **94**, 530.

† The pipettes were prepared by Dr. J. Francis Hartmann.