titratable –SH groups has been considered as a sign of denaturation. It seems, therefore, legitimate to interpret the changes just described as indications of a structural rearrangement of some protein molecules. Similar changes in –SH groups have been described in different systems; in the egg following fertilization(9) and in rhodopsin stimulated by light(10). The latter observation confirms Mirsky's hypothesis that light produces a reversible denaturation-like change in the protein moiety of visual purple(11). Other protein sidegroups are also "unmasked" in the process of denaturation, but the carboxyl or amino groups are not as readily detectable as the –SH groups. Ionization of the phenolic hydroxyl of tyrosine was described earlier(7,4).

The possible role of changes of protein configuration in the mechanism of cellular excitation is fully discussed elsewhere(12). Reversibility is one of the essential conditions for any change to be directly associated with excitation. The problem of the comparatively slow restoration of protein structure, as compared with the prompt return of excitability has to be approached with more sensitive methods, capable of detecting changes caused by a small number of impulses. Another problem, that of the relation between protein changes and the shift of Na^+ and K^+ ions during excitation, is under investigation.

Summary. An increase in titratable –SH groups was observed in rat brain stimulated through its afferent nerves. The change is reversible when the resting state is restored. The increase produced by stimulation affects mainly the non-dialyzable, presumably protein-bound –SH groups. The change is interpreted as an indication of structural rearrangement of certain proteins which may participate in the mechanism of excitation.

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Received November 13, 1957. P.S.E.B.M., 1958, v97.

Pteroylglutamic Acid in Different Diseases. (23730)

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Sauberlich and Baumann(1) observed that pteroylglutamic acid (PGA) is converted to citrovorum factor (CF) in the body and the latter is the active form of the vitamin PGA. The metabolism of PGA has been studied in normal persons(2-4) and in patients suffering from liver diseases(5), pernicious anemia(6, 7), megaloblastic anemia(8), gout(9), cancer and tuberculosis(10). Considering the various functions of PGA in the different physiological and pathological processes in the animal body it was of interest to study the metabolism of PGA in patients suffering from various diseases and in normal persons, by estimating urinary excretions of PGA and CF.

Methods. 17 cases of cirrhosis of liver, 10 of typhoid fever with positive blood culture,

TABLE I. 24-Hour Urinary Excretion of PGA and CF by Normal Subjects and by Patients Suffering from Various Diseases.

			Supplements		
		Nil	10 mg PGA (mouth)	10 mg PGA+ 500 mg ascorbic acid (mouth)	10 mg PGA (intrav.)
Normal (10)	PGA	4.2 ± .1*	4490 ± 137	4583 ± 147	7588 ± 62
	CF	.4 ± .0	14.0 ± .6	28.3 ± 1	14.1 ± .1
Cirrhosis of liver (17)	PGA	2.7 ± .1	2611 ± 95	2662 ± 96	4837 ± 240
	CF	.2 ± .1	6.2 ± .3	12.2 ± .5	6.9 ± .2
Typhoid fever (10)	PGA	1.4 ± .2	2984 ± 126	3128 ± 113	7450 ± 369
	CF	.3 ± .1	4.8 ± .3	10.1 ± .7	5.3 ± .4
Renal hypertension (10)	PGA	3.5 ± .1	3342 ± 212	3440 ± 67	7540 ± 386
	CF	.1 ± .0	4.2 ± .2	7.0 ± .1	4.5 ± .3
Essential hypertension (5)	PGA	4.0 ± .3	4492 ± 241		
	CF	.4 ± .0	12.6 ± .3		
Acute malaria (5)	PGA	3.1 ± .3	4541 ± 450		
	CF	.3 ± .0	4.6 ± .3		
Influenza (5)	PGA	3.0 ± .3	4697 ± 700		
	CF	.3 ± .0	6.5 ± .8		
Tuberculosis (5)	PGA	4.3 ± .2	4458 ± 206		
	CF	.4 ± .0	13.0 ± .9		
Nutritional anemia (10)	PGA	3.1 ± .2	3170 ± 128		6140 ± 135
	CF	.2 ± .0	7.0 ± .3		7.5 ± .4

* Mean ± S.E. Figures in parentheses indicate No. of subjects.

10 of renal hypertension, 5 of essential hypertension, 5 of acute malaria, 5 of influenza, 5 of tuberculosis and 10 of nutritional anemia were selected from patients admitted into the Nilratan Sircar Medical College Hospitals, Calcutta. Ten normal subjects selected for investigation were research students of Presidency College, Calcutta belonging to the same economic and social status as the selected patients. 24-hour output of urine of each subject was collected in bottles under toluene for 3 days consecutively. Each subject was then fed 10 mg PGA and urine was again collected for 3 days consecutively. PGA and CF excretions were determined in the different urine samples. When these excretions became normal the subjects were fed 10 mg PGA and 500 mg ascorbic acid. Urine samples were collected as before and excretions of PGA and CF determined. These excretions were also determined after intravenous injections of 10 mg PGA. PGA was estimated by the differential microbiological assay method(11) *using Streptococcus fecalis R*, CF was determined (1) using *Leuconostoc citrovorum* 8081 as test organism. The results are given in Table I.

Results. Cirrhosis of liver. Patients suffering from cirrhosis of liver excreted in urine lesser amounts of PGA and CF than normal persons both before and after feeding of PGA. This is in agreement with the observations of Oji *et al.*(5). When PGA was injected intravenously CF excretions did not differ from the excretion after PGA was fed. This indicated that the diminished excretion of CF was not due to defective intestinal absorption of PGA. Nichol(12) showed that liver was the principal site of conversion of PGA to CF. In cirrhosis of liver the parenchymatous tissues are replaced by fibrous tissues. It is, therefore, possible that due to inadequate conversion of PGA to CF the excretion of the latter was diminished. Lesser excretion of PGA was possibly due to its destruction or its retention in the tissues.

Typhoid fever. Excretions of PGA and CF before and after feeding of PGA with or without ascorbic acid supplement also diminished in similar manner as the patients suffering from cirrhosis of liver. After intravenous injection of PGA excretion of CF did not increase as compared to excretion when PGA was fed. This indicated that although there was lesion in the small intestine this did not interfere with the absorption of PGA to CF (13). Less excretion of CF after PGA was fed might, therefore, be due to less conversion of PGA to CF in typhoid fever. The di-

minished excretion of PGA might be due to its destruction by the typhoid bacillus as it has been suggested(14) that pathological invasion of small intestine by bacteria might be responsible for the destruction of PGA. When PGA was administered intravenously so as to avoid the intestinal route, excretion of PGA was similar to excretions by normal subjects, which further supports the contention that PGA is destroyed in the intestine by the typhoid bacillus.

Renal hypertension. Patients suffering from renal hypertension also excreted diminished amounts of PGA and CF. Intravenous administration of PGA induced increased elimination of PGA as under similar conditions in normal persons but the excretion of CF did not increase as compared to that when PGA was fed. Kidney, therefore, might be responsible for the conversion of PGA to CF.

Acute malaria and influenza. Patients suffering from acute malaria and influenza excreted significantly less amounts of PGA and CF before administration of folic acid. When PGA was fed excretion of PGA was greater and the excretion of CF was less than the excretion by normal men. In these diseases, therefore, due to fever metabolic disturbances might interfere in the conversion of PGA to CF.

Nutritional anemia. In patients suffering from nutritional anemia the red blood cells were hyperchromic and macrocytic. These patients also excreted diminished amounts of PGA and CF both before and after PGA was fed. When PGA was injected intravenously while excretion of PGA was increased, excretion of CF did not change. Conversion of PGA to CF was, therefore, disturbed in these patients. Similar disturbance in PGA metabolism was also observed in pernicious anemia(6) and megaloblastic anemia(8).

The above observations indicate that the different tissues of the body are concerned in the conversion of PGA to CF, and in the diseased conditions studied, more PGA or preferably CF should be administered.

Summary. 1) Urinary excretions of PGA and CF were estimated in patients suffering from cirrhosis of liver, typhoid fever, renal hypertension, essential hypertension, acute malaria, influenza, tuberculosis, nutritional anemia and in normal subjects. They were also studied after administration of PGA by both enteral and intravenous routes, with or without simultaneous administration of ascorbic acid. Patients suffering from cirrhosis of liver, typhoid fever, renal hypertension, acute malaria, influenza and nutritional anemia excreted diminished amounts of PGA and CF than normal persons. After feeding of PGA patients suffering from above diseases excreted less CF than normal persons under similar conditions. These excretions in patients suffering from essential hypertension and tuberculosis were similar to excretions by normal persons. Administration of ascorbic acid along with PGA enhanced urinary excretion of CF in all diseased conditions but the increase was less than that observed in normal subjects. Intravenous injection of PGA increased the output of PGA in urine but excretion of CF was similar to excretions observed after feeding PGA. 2) Different tissues of the body seem to be concerned in conversion of PGA to CF in the body and administration of PGA or CF in different diseased conditions is suggested.

We are grateful to Dr. A. K. Datta Gupta, Principal, Nilratan Sircar Medical College hospitals, Calcutta for kind permission to use patients of the hospital. PGA, leucovorin and culture of *Leuconostoc citrovorum* were kindly supplied by Lederle Laboratories (India) Ltd.

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Received November 14, 1957. P.S.E.B.M., 1958, v97.

Occurrence of Porphyrins in Peripheral Nerves.* (23731)

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The occurrence of porphyrins in the central nervous system of a wide variety of animals was first described by Klüver(1). In his extensive survey of the nervous system of a large number of vertebrates, he(2) was able to demonstrate that the white matter of the central nervous system of warm-blooded animals contained coproporphyrin. Later, he extracted protoporphyrin in variable amounts. Klüver's spectroscopic examination of the peripheral nerves revealed that porphyrins were present in the optic, trigeminal, facial, and auditory nerves(1,2). He was unable, however, to detect the presence of porphyrins in the occulomotor group of nerves (III, IV, VI) or in spinal nerves. Further quantitative and qualitative study of the porphyrins in the central nervous system(3,4) confirmed Klüver's observations and it became apparent that the porphyrin in the central nervous system was a mixture of coproporphyrin and protoporphyrin. The discovery that the coproporphyrinuria in patients with poliomyelitis involved a type III porphyrin(7) focused attention on the coproporphyrin III concentration of the nervous system. Blanchard isolated and crystallized the coproporphyrin from beef brains and established that this was the type III isomer on the basis of the melting point of the methylester. It was early observed by one of us that the red fluorescence of the porphyrins in the nervous system was not uniformly distributed. Quantitative determinaton revealed a higher concentration of coproporphyrin III in the medulla oblongata and brain stem than in cerebellum, cerebrum or spinal cord. Since porphyrin was abundant in the conduction areas of the central nervous system, and was found to be most concentrated in the important medullary region, it was postulated that the porphyrins may be involved in the process of conduction of nerve impulses. However, such a hypothesis would be untenable if porphyrins were absent in peripheral nerves. It was, therefore, decided to determine whether porphyrin was present in peripheral nerves and in what amounts. It was found that both protoporphyrin and coproporphyrin were relatively abundant in peripheral nerves. Moreover, it was observed that the peripheral nerves contain approximately 2 molecules of coproporphyrin to each molecule of protoporphyrin.

Methods and materials. The lumbo-sacral trunk and portions of the sciatic nerve were removed from the pigs immediately after they had been slaughtered. The nerves were transported to the laboratory in plastic bags packed in salted, crushed ice. They were separated from fat and blood vessels, washed in distilled water to remove blood, and either used immediately or wrapped in Saran and stored in the deep freeze. For each determination, 150 g quantities of nerves were homogenized in a blendor with 500 ml of 4:1 ethyl acetate-glacial acetic acid for 20 to 30 minutes. The mixture was placed in the deep freeze until the temperature reached 6°C and was then filtered through a Büchner funnel. The filtercake was extracted with cold ethyl acetate-glacial acetic acid (4:1) until the filtrate became colorless. The porphyrins were

* Aided by grants from Anna Fuller Fund, Am. Cancer Soc., Md. Div., and Div. of Allergy and Infectious Diseases, P. H. S.

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