STUDIES ON THE SITE OF CONVERSION OF PTEROYL GLUTAMIC ACID TO CITROVORUM FACTOR IN RATS

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It was observed by Sauberlich (1949) that administration of pteroyl glutamic acid (PGA) in rats produced an increased urinary excretion of a factor required for the growth of the organism, *Leuconstoc citrovorum* 8061. The factor was termed *citrivorum factor* (CF) and was identified by Pohland *et al.* (1951) as a reduced and formylated derivative of PGA, N^5-formyl tetrahydro PGA. Guggenheim *et al.* (1956) observed that urinary excretions of PGA and CF increased considerably after feeding PGA to rats and the increase was further enhanaced by the addition of ascorbic acid to PGA. Black *et al.* (1941) observed that when rats were fed with a synthetic diet containing 0.5 per cent sulfaguanidine the animals grew at a very slow rate. Nielsen and Elvehjem (1942) reported that PGA counteracted the growth inhibition of sulfathiazole, and Dietrich *et al.* (1950) were of the opinion that PGA and vitamin B_{12} were synthesized by the intestinal micro-organisms which led to the increased accumulation of these vitamins in the liver. Ferrari (1955) reported that rats injected with carbon tetrachloride excreted less CF than normal rats after an intraperitoneal injection of PGA, although the total PGA activity of the urine showed no significant difference between the rats differently treated. Nichol and Welch (1950) observed that liver was the principal site of conversion of PGA to CF in the body and an enzyme system seems to be responsible for maximum conversion of PGA to CF (Doctor *et al.*, 1954).

The present communication deals with the studies carried out to ascertain the site of conversion of PGA to CF *in vivo* and *in vitro*.

EXPERIMENTAL

Male rats of weights varying between 150 and 200 gm. were used. The animals were fed with mineralised (Schantz *et al.*, 1938) whole milk with two drops of a concentrate of vitamin A and D, twice a week. The rats were housed in metabolism cages and 24-hour urine samples were collected in conical flasks under toluene for consecutive three days and urinary excretions of PGA and CF were estimated. The rats were then fed with 100γ PGA for consecutive three days by stomach tube and urine samples were collected every day. After a few days when the urinary excretions of PGA and CF reached normal levels, the rats were given 100γ PGA by intraperitoneal injections for consecutive three days and daily urine samples were collected. When the excretions of PGA and CF reached normal values, the animals were fed with 125 mg. sulfasuxidine for consecutive three days by stomach tube and then a supplement of 100γ PGA together with 125 mg. sulfasuxidine were introduced by stomach tube and the urinary samples collected as before. The animals were then fed with 31.25 mg. chloromycetin palmitate for three days followed by a supplement of 100γ PGA along with chloromycetin for another three days.

The rats were then given intraperitoneal injections of carbon tetrachloride (0.03 c.c./100 gm. body wt.) for ten days and when the animals showed fall in weight and aversion for food they were given intraperitoneal injections of 100γ

PGA for three days and the injections of carbon tetrachloride were continued, Urinary excretions of PGA and CF were estimated in the different urine samples throughout the experimental period. PGA was estimated by the differential microbiological assay method described by Weiland *et al.* (1952) using *Streptococcus faecalis* R. as the organism and CF was determined using *Leuconstoc citrovorum* 8081 by the method of Sauberlich and Baumann (1948).

The results are given in Table I.

TABLE I

Average 24 hours urinary excretions of pteroyl glutamic acid (PGA) and citrovorum factor (CF) by rats fed with different supplements

Supplement	PGA (γ)	CF (γ)
None (8)	*1.04±0.08	0.077±0.003
PGA[1](8)	10.40±0.63	0.455±0.023
Sulfasuxidine[2](4)	0.49±0.06	0.058±0.007
Sulfasuxidine[2](4) + PGA	14.73±1.70	0.343±0.041
Chloromycetin palmitate[3](4)	0.49±0.05	0.050±0.004
Chloromycetin palmitate (4) + PGA	18.97±1.13	0.326±0.001
PGA[4] injection (8)	29.30±4.05	0.413±0.025
CCl_4[5] injection (7)	0.53±0.05	0.042±0.003
CCl_4[5] injection + PGA injection.	31.40±2.54	0.416±0.024
CCl_4 injection + PGA[1] (4)	9.40±0.30	0.335±0.03

[1] 100γ PGA fed per animal per day for 3 days.
[2] 125 mg. sulfasuxidine fed per animal per day for 3 days.
[3] 31.25 mg. chloromycetin palmitate fed per animal per day for 3 days.
[4] 100γ PGA injected intraperitoneally per animal per day for 3 days.
[5] 0.03 c.c./100 mg. body weight CCl_4 injected intraperitoneally per animal per day for 10 days.
Figures in parenthesis indicate the number of animals.
* Mean ± Standard Error.

At the end of the experiment the carbon tetrachloride treated rats and normal rats were killed after an over-night fast by decapitation. Liver, intestine, kidney, brain, spleen and pancreas were removed, chilled, adherent blood soaked and portions of the tissues were weighed. A 20 per cent homogenate of the different tissues were prepared with ice cold 0.08 M sodium phosphate of *p*H 6.3 and 5 c.c. of the homogenate were taken in 50 c.c. conical flasks. To each flask 5 c.c. of phosphate buffer of *p*H 6.3, 1 c.c. of a solution containing 100γ PGA and 1 c.c. toluene were added. The control flask contained 1 c.c. of carbon dioxide free water in place of PGA. Air in the flasks were replaced by a current of nitrogen, flasks stoppered, incubated for 4 hours at 37°C, stoppers replaced by cotton wool plugs, autoclaved for 5 minutes at 10 lbs. pressure, contents transferred to 100 c.c. volumetric flasks, neutralised to *p*H 6.8 with sodium hydroxide solution with bromothymol blue as external indicator, diluted to 100 c.c. with water and filtered. An aliquot of the filtrate was diluted suitably for the determination of PGA and CF by the microbiological assay method as used in these estimations in urine samples.

Percentage of PGA converted to CF and percentage of PGA destroyed, if any, by the different tissues were calculated and the results are presented in Table II.

TABLE II

Conversion of PGA to CF and destruction of PGA by the tissues of rats when 1 gm. tissue is incubated with 100γ PGA in phosphate buffer of pH 6.3 in an atmosphere of nitrogen for 4 hours (average of 7 observations).

Tissues	Conversion of PGA to CF (%)		Destruction of PGA (%)	
	Normal	CCl_4-treated	Normal	CCl_4-treated
Liver	4.82±0.20	0.06±0.01	17.4±0.7	33.8±4.4
Small intestine	0.03±0	0.01±0	6.9±0.9	8.0±1.1
Kidney	0.04±0	0.04±0	10.0±1.0	12.9±0.9
Brain	0.01±0	0.01±0	9.4±1.1	10.7±1.0
Spleen	0.03±0	0.02±0	10.3±1.3	14.6±1.3
Pancreas	0.02±0	0.02±0	4.7±0.5	10.5±0.9

DISCUSSION

Increased urinary excretions of PGA and CF were observed in rats fed with PGA. This was in confirmation of the works reported by other workers (Guggenheim *et al.* 1956). Comparatively less excretion of CF, however, was observed when PGA was injected intraperitoneally. This was possibly due to rapid rate of absorption of PGA and its distribution in the general circulation so that less time was taken for the conversion of PGA to CF by the tissues.

When the rats were fed with sulfasuxidine the urinary excretions of PGA and CF diminished. Intestinal bacteria synthesises PGA and sulfasuxidine retards their growth (Dietrich *et al.*, 1950). As less PGA is synthesised after sulfasuxidine less amount of it is converted into CF leading to its diminished excretion in urine. When PGA was fed to rats treated with sulfasuxidine there was increased excretion of PGA than the normal animals fed with PGA but the excretion of CF was lesser than the normal excretion. This indicates that possibly intestinal flora plays some rôle in the conversion of PGA to CF. Chloromycetin palmitate behaves in the similar way as sulfasuxidine thus emphasising the possible rôle of intestinal flora in the conversion of PGA to CF.

After injection of carbon tetrachloride the urinary excretion of PGA and CF greatly diminished. Carbon tetrachloride-treated rats excreted lesser amounts of both PGA and CF in urine after they were fed with PGA. Lesser excretion of PGA might be due to its destruction and diminished excretion of CF was possibly due to the diminished conversion of PGA to CF in the liver which is damaged by carbon tetrachloride. The intraperitoneal injection of PGA in carbon tetrachloride treated rats did not produce any apparent change in the urinary excretions of PGA and CF which was unlike the observation of Ferrari (*loc. cit.*). This might indicate that tissues of the body other than the liver are also concerned in the PGA metabolism in the body.

In vitro studies with different tissues of normal rats indicated that liver was mainly concerned in the conversion of PGA to CF. The other tissues studied,

namely small intestine, kidney, brain, spleen and pancreas played insignificant rôle in the conversion. When tissue homogenates were incubated with PGA it was not recovered completely which possibly indicated its destruction. The maximum destruction of PGA was observed with the liver and the destruction by other tissues was in the following order—kidney, spleen, brain, small intenstine and pancreas.

Carbon tetrachloride-treated rat tissues could convert PGA to CF less efficiently. The conversion of PGA to CF was very much depressed in the liver and small intestine. Destruction of PGA was also greater with the tissues of carbon tetrachloride treated rats. Although liver has been found to be the principal tissue concerned in the conversion of PGA to CF, destruction of PGA was also maximum with the liver tissue. Tissues might contain some enzyme which helps in the disintegration of PGA. Liver, small intestine and intestinal flora seem to be primarily concerned in the transformation of PGA to CF.

Summary

Daily urinary excretions of PGA and CF were determined in normal rats, in rats fed with sulfasuxidine and chloromycetin and in rats injected with carbon tetrachloride both before and after administration of PGA.

Urinary excretions of PGA and CF diminished when the rats were fed with sulfasuxidine and chloromycetin and also when they were treated with carbon tetrachloride.

Different tissues e.g., liver, small intestine, kidney, pancreas, spleen and brain from normal and carbon tetrachloride treated rats were incubated with PGA for 4 hours and PGA and CF were estimated.

More CF was formed by liver and small intestine from normal rats while the other tissues had practically no effect in this conversion, and this conversion was greatly affected in the liver and small intestine from carbon tetrachloride treated rats.

Different tissues of normal rats destroyed PGA and the destruction was maximum with the liver and least with the pancreas. Tissues from carbon tetrachloride treated rats destroyed PGA to a greater degree.

Liver, small intestine and intestinal flora seem to be concerned in the conversion of PGA to CF.

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