TABLE I. Studies with Synthetic Folinic Acid.

Trial	Group	Supplement/kg of basal ration SIF	Avg wt, g, 4 wk	Feed efficiency*
1	1	0	120	2.63
	2	.5 mg folinic acid	226	2.01
	3	.5 mg PGA	231	1.96
	4	30 mcg cryst. vit. B_{12}	127	2.51
	5	1 mg cryst. vit. B_{12}	142	2.33
	6	2 mg PGA	269	2.13
			26 days	
2†	1	0	106	2.66
	2	.1 mg folinic acid	126	2.57
	3	.1 mg PGA	105	3.08
	4	.4 mg folinic acid	209	2.09
	5	.4 mg PGA	167	2.21
	6	2 mg PGA	211	2.13

* Feed efficiency—Total feed consumed / wt gained

† Trial 2—30 mcg cryst. vit. B_{12}/kg added to basal ration.

which has been reported to spare PGA(9).

In trial 2, two different levels of folinic acid were compared to similar levels of PGA. The growth responses and feed efficiency figures show that the folinic acid was fully as active as PGA; whether it may be even more active will require more work to fully determine.

It is clearly evident that folinic acid should be added to the list of known compounds of the folic acid group which are active for the chick.

Summary. Studies on the biological activity of synthetic folinic acid show that it may replace pteroylglutamic acid in the diet of the chick.

9. Shaefer, A. E., Salmon, W. D., Strength, D. R., and Copeland, D. H., *J. Nutr.*, 1950, v40, 95.

Received December 19, 1950. P.S.E.B.M., 1951, v76.

Effect of Administration of ACTH and Cortisone upon Blood Glutathione Levels (18510)

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Conn and his associates(1,2) reported that the administration of ACTH resulted in diminished glucose tolerance and glycosuria which were associated with a decrease of blood glutathione (GSH) and an increased excretion of urinary uric acid. Conn, Lewis, and Johnston(3) showed that the administration of GSH to a normal individual receiving ACTH produced a dramatic but transitory reduction in the hyperglycemia and glycosuria. This observation, as Conn *et al.*(1) have indicated, is of interest inasmuch as the diabetogenic activity of alloxan, a possible purine metabolite, can be decreased by GSH as well as other substances containing the thiol group. Kinsell *et al.*(4) found an increased urinary excretion of sulfur-containing compounds following the administration of ACTH. Lazarow and Berman(5) noted that the injection of cortisone into normal rats led to a decrease in blood GSH which appeared to correlate with the degree of glycosuria. Lazarow (6) found that GSH potentiated the action of cortisone and, in contrast to its action during ACTH therapy reported by Conn *et al.* (3), produced an increased glycosuria when injected into cortisone diabetic rats.

The observations of Conn *et al.*(1,2) upon

* Lt. j.g. (M.C.) U.S.N.

1. Conn, J. W., Louis, L. H., and Wheeler, C. E., *J. Lab. and Clin. Med.*, 1948, v33, 651.

2. Conn, J. W., Louis, L. H., and Johnston, M. W., *J. Lab. and Clin. Med.*, 1949, v34, 255.

3. Conn, J. W., Louis, L. H., and Johnston, M. W., *J. Clin. Invest.*, 1949, v28, 775.

4. Kinsell, L. W., Li, C. H., Margen, S., Michaels, C. D., and Hedges, R. N., Proc. of the First ACTH Conference, Blakiston Co., Phila., 1950, p. 70.

5. Lazarow, A., and Berman, J., *Anat. Rec.*, 1950, v106, 215.

6. Lazarow, A., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 702.

TABLE I. Blood Glutathione (GSH) and Total Glutathione (GSH and GSSH) and Blood Glucose Following ACTH.

No.	Control	mg %	During administration of ACTH mg %	mg %	mg %	mg %	Max. reduction in GSH, %
1	GSH	40.9	*37.3*(2)*	42.4(5)	39.3(7)	37.8(10)	9
	Total	50.8	53	47.1	56.3	54.6	
	Glucose	95	106		112		
2	GSH	47.0	47.1(1)	46.2(2)	*24.6*(6)	35.4(8)	48
	Total	63.2	64.7	60.1	62.1	53.9	
	Glucose	91		131	106	103	
3	GSH	33.9	*24.6*(1)	27.7(5)	27.7(8)	39.8(11)	28
	Total	47.6	33.9	43.1	38.5	45.6	
	Glucose	95	97	106	94	95	
4	GSH	44.6	33.9(1)	32.3(2)	*27.7*(4)	38.5(6)	39
	Total	48.0	43.1	43.1	42.0	46.2	
5	GSH	43.1	40.0(2)	43.1(4)	*31.6*(8)	46.2(18)	26
	Total	46.2	67.8	67.8	70.8	55.4	
	Glucose	95		105		105	
6	GSH	32.3	*30.8*(2)	38.6(3)	43.1(5)	40.0(9)	5
	Total	53.3	47.7	46.2	52.4	46.2	
	Glucose	100	107	96	112	95	
7†	GSH	33.1	24.6(2)	*20.0*(5)	26.1(10)	26.1(15)	40
	Total	40.8	38.5	37.0	37.0	35.4	
	Glucose	113	126	138		128	
8	GSH	34.0	*26.2*(1)	34.5(3)	38.5(5)	33.4(7)	23
	Total	56.2	45.6	44.6	52.4	52.4	
	Glucose	102	102	107	95	96	
9	GSH	31.4	34.4(1)	31.4(4)	*23.6*(6)	28.3(8)	25
	Total	44.0	45.6	43.1	38.6	46.3	
	Glucose	97		114	100		
10	GSH	32.2	*22.0*(1)	25.1(3)	34.9(4)		32
	Total	46.3	40.0	41.1	45.0		
	Glucose	99	117	104			
11†	GSH	30.4	*21.6*(2)	21.6(4)	33.9(7)	27.7(9)	29
	Total	50.0	55.0	40.0	46.2	46.2	
	Glucose	97	129	121	109	115	
Avg	GSH	36.6 ± 6.0		26.8 ± 5.5		$t = 4.23$	
	Total	49.7 ± 6.0		47.9 ± 11.4		$t = 0.50$	

* No. in parentheses = No. of days on ACTH.
† Glycosuria developed on therapy.

the lowering of blood GSH levels following the injection of ACTH were made upon four patients and have been confirmed, in one case, by Kass, Ingbar, and Finland(7). Sprague *et al.*(8) followed the blood GSH in three patients who received both ACTH and cortisone and although the GSH showed little alteration with ACTH a slight increase in concentration of GSH occurred during cortisone therapy. We have determined GSH and total glutathione (GSH plus GSSG) in the blood following the administration of both ACTH and cortisone and have also determined blood sugar quantitatively and urine sugar qualitatively.

Experimental. Reduced glutathione (GSH) and total glutathione (GSH plus GSSG) were determined by the iodometric method of Hess (9) as modified by Woodward and Fry(10). This method has been criticized for a lack of specificity because both ergothioneine and ascorbic acid will react with iodine. As Hess (9) pointed out ergothioneine, present in

7. Kass, E. H., Ingbar, S. H., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 669.

8. Sprague, R. G., Power, M. H., Mason, H. D., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Arch. Int. Med.*, 1950, v85, 199.

9. Hess, W. C., *J. Wash. Acad. Sci.*, 1929, v19, 419.

10. Woodward, G. E., and Fry, E. G., *J. Biol. Chem.*, 1932, v97, 465.

TABLE II. Blood Glutathione (GSH) and Total Glutathione (GSH and GSSH) and Blood Glucose Following Cortisone.

No.	Control	mg %	During administration of cortisone mg %	mg %	mg %	mg %	Max. reduction in GSH, %
1	GSH	30.5	34.1(3)*	*25.1*(10)			18
	Total	46.1					
	Glucose	85					
2	GSH	27.8	*15.7*(2)	22.9(7)			43
	Total	48.6	39.3	47.7			
	Glucose	115	114	110			
3	GSH	33.0	19.8(1)	*18.8*(4)	34.4(11)		40
	Total	55.4	52.8	41.8	45.6		
	Glucose	105		98	114		
4	GSH	34.9	*26.7*(7)				20
	Total	41.9	43.0				
5	GSH	47.1	40.4(1)	*36.9*(2)	42.4(3)	44.0(5)	21
	Total	59.9	51.1	49.1	51.9	54.6	
	Glucose	97		104	102	106	
6	GSH	42.3	44.7(2)	30.8(6)	*27.7*(9)	30.8(18)	34
	Total	61.8	58.5	49.3	50.8	48.3	
	Glucose	90		89			
7	GSH	40.0	47.7(4)	*38.5*(5)			4
	Total	61.6	55.4	61.6			
	Glucose		107	110			
8	GSH	50.8	53.9(1)	*46.2*(3)	57.0(7)	55.4(10)	9
	Total	63.1	66.2	67.8	72.6	68.3	
	Glucose	101		91			
9	GSH	26.2	44.7(1)	*35.4*(6)			(increase = +35)
	Total	47.7	59.2	55.0			
	Glucose	95	107	99			
10	GSH	39.3	*35.5*(1)	43.3(2)			10
	Total	52.3	44.5	56.6			
	Glucose	120	110	95			
11	GSH	28.6	36.9(1)	37.2(2)	*31.4*(3)	37.4(4)	(increase = +8)
	Total	42.3	47.1	45.7	51.2	48.6	
	Glucose	100	100	92	95		
12	GSH	19.3	*11.7*(3)	18.3(7)	29.7(15)		39
	Total	33.7	41.0	48.8	44.6		
	Glucose	102	103				
Avg	GSH	35.0 ± 9.0		29.1 ± 11.1	t = 1.42		
	Total	51.2 ± 9.9		48.9 ± 8.8	t = 0.62		

* No. in parentheses = No. of days on cortisone.

whole blood to the extent of only 7-8 mg per 100 cc, has a titer in this method of less than one-tenth of that of an equal weight of GSH and, consequently, cannot cause any significant error. The amount of ascorbic acid present in blood likewise is too small to affect the titer for GSH. The more specific amperometric method of Benesch and Benesch(11), as pointed out by them, gives values for blood GSH which are the same as those obtained by the method we have employed. ACTH and cortisone were administered in amounts that gave the usual physiological responses, eosinopenia, increased urinary excretion of 17 ketosteroids and so forth.

The changes which occurred with ACTH are given in Table I and those with cortisone in Table II. Blood glucose values are included in both tables. The control values are, in almost every instance, the average of two or more determinations upon fasting blood samples. The average GSH values for the control periods are substantially the same in both groups. The figure in parenthesis following each of the values obtained during the experimental periods is the number of days under treatment. The lowest GSH value

11. Benesch, R. R., and Benesch, R., *Arch. Biochem.*, 1950, v28, 43.

obtained in each series of determinations during treatment is italicized and the average GSH and total glutathione values together with their standard deviations are calculated from these data.

Following the administration of cortisone there was a drop in GSH to 29.1 mg per 100 cc of blood from the control value of 35.0 mg. However, the t value was 1.42 indicating that the decrease had a low order of significance. The administration of ACTH produced a more marked lowering of the GSH from the control value of 36.6 mg per 100 cc of whole blood to a value of 26.8 mg. There was no appreciable change in the total glutathione value after the administration of either ACTH or cortisone indicating that the decrease in the GSH was brought about mainly by its oxidation.

The blood sugar increased more than 10 mg per 100 cc of blood over the control values in eight of ten patients following the administration of ACTH. In three patients the highest blood sugar level occurred on the same day as the lowest GSH value and the highest figures for the other five patients were obtained, on the average, 1.2 days following the lowest GSH value. One patient, not included among those just discussed, with hyperinsulinism caused by hypertrophy of the islet cells (number 4 in Table I) had a control blood sugar level of 37 mg per 100 cc of blood which rose to 93 mg after four days of ACTH therapy. On this day the GSH concentration had dropped to 27.7 mg per 100 cc of blood from an initial value of 44.6 mg. After the discontinuance of treatment the GSH concentration returned to 38.5 mg per 100 cc and the blood sugar decreased to 33 mg. Following the administration of cortisone only two of eleven patients showed an increase in blood sugar of more than 10 mg per 100 cc over the control values. One of these demonstrated the highest blood sugar on the same day as the lowest GSH value but the other had the most marked hyperglycemia five days before the lowest GSH level was attained.

Glucose tolerance tests were conducted on four patients before and during ACTH therapy. Two of these patients had mild diabetic patterns prior to ACTH administration but showed no further impairment of carbohydrate tolerance during therapy. The average decrease in their blood GSH was 16 percent. The other two patients developed typical diabetic glucose tolerance curves during ACTH therapy whereas their curves were normal prior to treatment. The average decrease in blood GSH was 38 percent. The number of patients is too small to draw any conclusion but the results do indicate that the change in glucose tolerance and GSH may be related. They also suggest, contrary to expressed opinion(8), that although individuals with normal glucose tolerance may show impairment of carbohydrate metabolism during adrenal stimulation those with mildly decreased tolerance demonstrate no further inability to handle glucose.

Glucose tolerance tests were conducted on four patients receiving cortisone. In two cases the curves were mildly diabetic before therapy and showed no change during treatment, the decrease in GSH averaging 5.5 percent. In one patient the curve was normal before cortisone but became typically diabetic during treatment and the GSH decreased 14 percent. The last patient had normal glucose tolerance curves before and during cortisone therapy and the blood GSH actually increased 35 percent.

A further indication of the relationship between the blood glucose level and the decrease in blood GSH following ACTH may be noted by correlating the increase in blood glucose with the decrease in GSH. As shown in Table I the five subjects with the greatest increase in blood sugar following ACTH therapy showed an average decrease in GSH of 34.8 percent whereas the five subjects with the least increase in blood glucose gave an average decrease in GSH of only 19 percent.

Summary. Blood glutathione (GSH) and total glutathione (GSH plus GSSG) were determined in eleven patients during ACTH therapy and in eleven patients during the administration of cortisone. GSH values were significantly decreased from a pretreatment average of 36.6 mg per 100 cc to 26.9 mg by ACTH while cortisone produced a decrease

from an average pretreatment value of 35.0 mg per 100 cc to 29.1 mg. The latter decrease, statistically, is of a low order of significance. The total glutathione values showed no changes following the administration of either ACTH or cortisone. During treatment with ACTH the greatest decrease in blood GSH occurred in the patients showing the greatest increase in blood sugar. There is suggestive evidence that although patients with normal glucose tolerance may show diabetic patterns during treatment, those who demonstrate mildly diabetic patterns prior to therapy show no further decrease in glucose tolerance and minimal reduction in GSH values after ACTH or cortisone therapy.

Received December 26, 1950. P.S.E.B.M., 1951, v76.

Inhibition of Citric Acid Synthesis *in vivo* by X-irradiation.* (18511)

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Previous investigations(1-4) have shown that the activity of a number of enzymes is markedly inhibited by ionizing radiations *in vitro*. However, the extent to which enzyme inactivation is involved in the acute toxic effects of ionizing radiations has not yet been clearly established. Thus, further studies on the biochemical changes in tissues taken from irradiated animals are indicated in order to find which enzymatic reactions are affected by ionizing radiations in the intact animal. The recent introduction by Potter(5) of the concept of sequential blocking of metabolic pathways has provided a new approach for studying the actions of toxic agents on intermediary carbohydrate metabolism *in situ*. This procedure consists of the use of an inhibitor known to block a reaction in the tricarboxylic acid cycle with the resultant accumulation of one of the Krebs intermediates in concentrations high enough to allow quantitative measurements. Sodium monofluoroacetate was employed by Potter(5) for this purpose. Buffa and Peters(6,7) demonstrated that this compound causes an accumulation of citric acid in rat tissues and subsequent investigations(8-11) have shown that this effect is due to inhibition of the oxidation of citric acid probably through prior conversion(8,9,12) of fluoroacetate to fluorocitrate or a related compound. Detailed studies by Potter, Busch, and Bothwell(13) have established standard conditions for use of fluoroacetate as an inhibitor of citrate oxidation *in vivo*.

In the present investigation the technic of sequential blocking(5) was employed to study the effects of X-ray on the operation of the

* The work described in this paper was conducted at the University of Chicago Toxicity Laboratory under a research contract supported by the Atomic Energy Commission.

1. Dale, W. M., *Biochem. J.*, 1940, v34, 1387.

2. Dale, W. M., *Biochem. J.*, 1942, v36, 80.

3. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.*, 1949, v32, 537.

4. Barron, E. S. G., and Dickman, S., *J. Gen. Physiol.*, 1949, v32, 595.

5. Potter, V. R., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 41.

6. Buffa, P., and Peters, R. A., *Nature*, 1949, v163, 914.

7. Buffa, P., and Peters, R. A., *J. Physiol.*, 1950, v110, 488.

8. Liebecq, C., and Peters, R. A., *Biochim. Biophys. Acta*, 1949, v3, 215.

9. Elliott, W. B., and Kalnitsky, G., *Fed. Proc.*, 1950, v9, 168.

10. Potter, V. R., and Busch, H., *Fed. Proc.*, 1950, v9, 215.

11. Potter, V. R., and Busch, H., *Cancer Research*, 1950, v10, 353.

12. Elliott, W. B., and Kalnitsky, G., *J. Biol. Chem.*, 1950, v186, 487.

13. Potter, V. R., Busch, H., and Bothwell, J., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 38.