represents only 1% of the total body weight of a pigeon, the changes in phosphorus turnover in the crop-sac may not be expected to influence appreciably the large reservoir of phosphate present in the extracellular fluid. The data suggest that inorganic phosphorus enters the cells of the stimulated gland at an increased rate and serves as the source of phosphate for the synthesis of organic phosphorus compounds. This is in conformity with the data to be presented below.

Prolactin produces an increase in the concentration of organic P^{31} in the acid-soluble fraction without influencing the specific activity. This suggests that prolactin increases the rate of synthesis of one or more of the phosphorylated intermediates included in this fraction. Prolactin increases the specific activity in the phospholipid fraction without changing its concentration of P^{31}. The role of phospholipid in the metabolism of fat is uncertain. The data could be interpreted to mean that phospholipid is involved (a) in the transport of fat to the crop-sac [see Chaikoff(9) for convincing evidence against such a role], or (b) in the intracellular synthesis and or oxidation of fat. It is of interest in this connection that both the cells(10) and the milk(11) of the crop-sac contain high concentrations of lipid material.

Prolactin increases the concentration of P^{31} in the residue fraction of the crop-sac without causing an associated change in specific activity. This suggests an increase in the synthesis of nucleoproteins; such an increase is expected in a gland which has undergone proliferation and is in agreement with the demonstration of McShan *et al.*(12) that prolactin increases the concentration of nucleic acids in the crop-sac.

Summary. Prolactin increases the concentration of total P^{31} and P^{32} in the pigeon crop-sac. The applicability of these changes to the bioassay of the hormone is discussed.

Prolactin (a) increases the specific activity without affecting the concentration of inorganic acid-soluble phosphorus in the crop-sac, (b) increases the concentration without affecting the specific activity of the organic acid-soluble fraction, (c) increases the specific activity without affecting the phosphorus concentration in the phospholipid fraction, and (d) increases the concentration without affecting the specific activity of phosphorus in the residue fraction. The significance of these results is discussed.

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Uric Acid Diabetes in the Rat.* (18583)

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An investigation into the susceptibility of the albino rat to uric acid diabetes was prompted by the recent reports of Griffith (1,2) that methionine deficient rabbits exhibited diabetic symptoms following the injection of uric acid. When rabbits, which re-

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ceived a complete ration (stock ration or the methionine deficient ration supplemented with that amino acid), were used no hyperglycemia resulted. Uric acid had been reported non-diabetogenic(3-5) but normal animals were used. The problem, therefore, resolved itself into testing the diabetogenic activity of uric acid in methionine deficient rats.

Methods and materials. Methionine deficient rats were obtained by feeding a ration composed of 18% protein (α-protein and oxidized casein),‡ 73% sucrose, 4% Salts IV (6), 5% corn oil and all the known vitamins. A similar ration which contained only 9% casein (81% sucrose) was also used. The control rations resulted from the supplementation with 0.3% dl-methionine (α-protein and 9% casein) and 0.6% dl-methionine (oxidized casein) and the use of 18% casein as the source of protein. When a sodium deficient ration was desired, the above salts were replaced by 2.5% sodium-potassium low Salts IV(7), the sucrose increased to 74.5% and the ration supplemented with 0.25% potassium as the bicarbonate. The raw soyabean ration was composed of 59% corn, 34% raw soyabean flakes, 5% alfalfa and 2% Salts IV. Male weanling rats (Holtzman) were placed on the experimental rations described above, for the lengths of time indicated in Tables I to IV. Then they were either subjected to injections of uric acid (Tables I and III) or their tissues were analyzed for glutathione (Tables II and IV). Those subjected to the uric acid treatment received an intraperitoneal injection of an aqueous suspension (75 to 350 mg/cc depending on the dosage). The blood sugar values were determined at 0, 2, 4 and 6 days after the initial injection of uric acid. The method of Folin and Malmros as adapted for the Evelyn colorimeter by Horvath and Knehr(8) was used. Successive uric acid injections were given as indicated in Tables I and III. The α-protein rats were fasted 12 hours before each bleeding and 15 hours before the uric acid injections. The sodium deficient rats were not fasted before the bleedings or uric acid injections. Blood, liver and kidney glutathione values were determined in all animals not subjected to uric acid injection according to the nitroprusside method as modified by Grunert and Phillips(9).

TABLE I. Effect of Intraperitoneal Injections of Uric Acid on Blood Sugar of Rats Fed a Methionine Deficient Ration (α-protein).

Time interval, days	Uric acid dosage, mg/kg	Blood sugar* α-protein, mg %	Blood sugar* α-protein + methionine, mg %
0	200	90 ± 10†	88 ± 7
2	—	92 ± 5	99 ± 4
4	500	106 ± 9	93 ± 6
6	—	97 ± 5	107 ± 8

* Fasting levels (12 hr).
† Stand. error of the mean, 4 rats.

Results. The report that α-protein was low in methionine(10) led to its use for producing methionine deficient rats. Two groups of rats (4 animals each) received as their protein supplement 18% α-protein and 18% α-protein plus 0.3% dl-methionine for 15 weeks. They were injected with uric acid at that time following a 15-hour fast. The blood sugar was determined before and at two day intervals after the injection. The results are shown in Table I. Two days after the intraperitoneal injection of 200 mg/kg of uric acid the blood sugar was unchanged. The use by Griffith of 1 g/kg in the rabbit(1,2) and the ratio of dosages of alloxan used in the rat

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‡ The α-protein was a purified protein fraction from soyabeans (Glidden Co., Chicago) which supplied 0.3% methionine when fed at 18% of the ration. In comparison, 18% casein supplies 0.6% methionine. The oxidized casein(13) ration was supplemented with 0.25% dl-tryptophane.

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TABLE II. Effect of a Methionine Deficiency, Induced by 9% Casein, α-protein and Oxidized Casein on the Blood and Liver Glutathione Levels of the Rat.

Ration	Time of exp., wk	No. of animals		Blood glutathione		Liver glutathione	
			.3% dl-methionine	mg %	.3% dl-methionine, mg %	mg/100 g	.3% dl-methionine, mg/100 g
9% casein	4‡	8	9	27.1 ± 2.5*	23.5 ± 1.7	43 ± 3	179 ± 7
18% α-protein	15	4	4	39.6 ± 2.6	34.3 ± 0.8	47 ± 9	126(2)
18% oxidized casein	2§	8	8	41.2 ± 2.8	41.6† ± 2.3	60 ± 4	109† ± 12
Raw soyabean meal	6	6	—	21.1 ± 0.7	—	138 ± 9	—
18% casein	6	27, 15	—	32.5 ± 0.8	—	111 ± 6	—

* Stand. error of the mean.
† Supplemented with .6% dl-methionine.
‡ Four weeks, following a 2-wk interval on 18% casein.
§ Two weeks, following a 4-wk interval on 18% casein.

TABLE III. Effect of Intraperitoneal Injections of Uric Acid on Blood Sugar Levels of Rats Fed a Sodium Deficient Ration.

Time interval, days	Uric acid dosage, mg/kg	Blood sugar*	
		Sodium deficient mg %	Control, mg %
0	250	—	—
2	500	139 ± 16†	128 ± 3
4	1000	121 ± 8	108 ± 1
6	—	123 ± 2	131 ± 5
0	1000	—	—
2	—	105 ± 7	115 ± 6

* Non-fasting levels.
† Stand. error of the mean, 4 rats.

and rabbit(11) led to the use of this level of uric acid. On the fourth day, with the blood sugar still normal a second injection of uric acid (500 mg/kg) was given without effect. There was apparent pain in the rats following the injection of uric acid, and this disappeared in a few hours.

The fact that uric acid appeared non-diabetogenic in the methionine deficient rat suggested that the tissue levels of glutathione may not have been lowered sufficiently. Accordingly the effect of the α-protein and other methionine deficient rations on blood and liver glutathione was studied. The results are shown in Table II. The liver glutathione of the rats which received the methionine deficient rations (9% casein, 18% α-protein and 18% oxidized casein) was decreased to less than 50% of the normal value of 111 mg/100 g. Supplementation of the deficient rations with methionine permitted the maintenance of normal levels of liver glutathione in the α-protein and oxidized casein rats and an increased level (179 mg/100 g) in the 9% casein rats. The blood glutathione reacted differently. It tended to vary with the type of ration used, as indicated in Table II. When α-protein and oxidized casein were fed the blood glutathione increased to 39.6 and 41.2 mg % respectively. It seems that low methionine rations cause elevated dl-methionine concentrations in the blood. The blood glutathione of rats maintained on α-protein for 7 months was 42.0 mg % compared to 32.1 mg % for the methionine supplemented rats, while the liver glutathione was 65.2 mg/100 g compared to 144 mg/100 g respectively.

The effect of uric acid on the blood sugar of sodium deficient rats was studied because of their low blood glutathione values(7,12) and the insistence of previous workers(2,13) for this criterion in developing uric acid diabetes. The results are shown in Table III.

The need for decreased levels of glutathione in both the liver and blood in order to demonstrate the diabetogenic activity of uric acid became apparent from the resistance of sodium deficient (low blood glutathione) and methionine deficient (low liver glutathione) rats to uric acid diabetes. An attempt was therefore made to lower the level of blood and liver glutathione simultaneously by super-

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TABLE IV. Blood, Liver, and Kidney Glutathione Levels as Affected by the Nutritional State of the Rat.

Nutritional state of rat	Tissue glutathione		
	Blood, mg %	Liver, mg/100 g	Kidney, mg/100 g
Control	32.5 ± 0.8*	111 ± 6	99 ± 6
Sodium deficient	15.3 ± 1.6	150 ± 9	98 ± 7
Methionine deficient (9% casein)	27.1 ± 2.5	43 ± 3	88 ± 2
" " (18% oxidized casein)	41.2 ± 2.8	60 ± 4	80 ± 4
Na + methionine (9% casein) deficient	17.2 ± 0.8	141 ± 9	107 ± 2
Na + methionine (oxidized casein) deficient	31.8 ± 1.7	58 ± 7	73 ± 6

* Stand. error of the mean, 8 to 10 rats per group except controls where 27 rats used for blood glutathione and 15 for liver and kidney glutathione.

imposing a methionine deficiency on top of a sodium deficiency. The results are shown in Table IV. The levels of kidney glutathione are included. The data from Table II on the effect of the 9% and 18% oxidized casein on blood and liver glutathione are also included for more direct comparison. As reported previously(12) the blood glutathione of sodium deficient rats was decreased (15.3 mg %) while the liver glutathione was elevated to 150 mg/100 g. When the 9% casein ration was superimposed on the sodium deficiency for the last 4 weeks of the 6 weeks experimental period the blood glutathione remained low at 17.2 mg % and liver glutathione remained elevated at 141 mg/100 g. When the oxidized casein ration was superimposed on the sodium deficiency for the last two weeks of the 6 weeks experimental period the blood glutathione returned to normal (31.8 mg %) and the liver glutathione decreased to 58 mg/100 g. The complete absence of methionine in oxidized casein(13) apparently decreased the requirement for sodium by virtue of a lack of growth and thereby eliminated the effect of sodium.

Discussion. The resistance of methionine deficient and sodium deficient rats to uric acid diabetes was thought at first to be in contradiction to the work of Griffith(1,2). The necessity for a low level of liver glutathione thus permitting the conversion of uric acid to some toxic agent (alloxan?) and a simultaneously low blood glutathione level to permit the passage of the agent to the pancreas was, however, realized. On this basis the sodium deficient rats were non-susceptible because of the normal metabolism of uric acid in the liver (normal glutathione content). The methionine deficient rats were resistant because of the high blood glutathione content which would undoubtedly tie up the toxic agent (alloxan?) formed by the sulfhydryl deficient liver.

The failure to obtain a nutritional state in which both the blood and liver glutathione levels were simultaneously reduced does not permit conclusive statements as to diabetogenic activity of uric acid in the rat. However it should be noted that Williams and Bailey(14) failed to confirm Griffith's work with rabbits. Furthermore, uric acid has been found to be converted quantitatively to allantoin by the sulfhydryl deficient livers from rats maintained on α-protein and oxidized casein(15). Because of this report it is felt that when and if a simultaneous lowering of blood and liver glutathione in the rat is obtained uric acid will still be non-diabetogenic.

Summary. Uric acid was non-diabetogenic in methionine and sodium deficient rats. Methionine deficiencies induced by 9% casein, α-protein and oxidized casein rations lowered the liver glutathione but did not lower the blood glutathione. The superimposing of a methionine deficiency on a sodium deficiency (low blood glutathione) did not cause a simultaneous decrease in the blood and liver glutathione of the rat.

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