Almost invariably action potentials in the reacting muscle were accompanied by corresponding ones in its antagonist. These antagonistic potentials might or might not correspond in time with the start of the reaction though it was usually within 10 sigma of being the same.

Wholly unexpectedly about 40% of all the records had an early volley of small discharges which appeared in as little as 25 sigma following the stimulus. The records from 3 of the subjects showed none of these early discharges and those from the remainder showed varying numbers, never, however, 100%. These early discharges, which were not accompanied by gross movement of the arm, occurred from 110 to 160 sigma earlier than the arm movement of the response. In the responding muscle they become larger, which augmentation was followed in 58 sigma (as above) by the first arm movement. These pre-response discharges were found in not only the responding muscle but also in its antagonist, in the triceps and biceps brachii of the contralateral arm which did not grossly move at all, and in the homolateral gastrocnemius. They are therefore quite widespread in their appeaarnce.

No complete theory can as yet be formulated in explanation of these pre-response discharges. The times of their appearance correspond roughly to the times for simple reflexes. It is possible that they originate in lower levels neurologically and so point to another relationship of the voluntary to the involuntary reaction. Further investigation is necessary in order to formulate more clearly the possibilities.

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Solubility of Calcium Oxalate and Uric Acid in Solutions of Urea.

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The high solubility of calcium oxalate and uric acid in urine has frequently been ascribed to the action of the colloids of urine.[1,2] Urea also is known to increase the solubility of a number of compounds. As the unsaturated amide of carbonic acid it combines with acids to

[1] Joly, J. S., Stone and Calcarous Diseases of the Urinary Organs, St. Louis, 1929.

[2] Pauli and Semac, *Biochem. Z.*, 1909, **17**, 235.

form salt-like compounds, many of which are highly soluble. Urea also forms addition compounds with salts, probably through the residual valence of its oxygen. The experiments recorded below are to ascertain how far urea may be responsible for the high solubility of calcium oxalate, uric acid and sodium urate in urine.

Crystalline calcium oxalate ($CaC_2O_4.H_2O$) was prepared by slowly running 0.250 N $CaCl_2$ and 0.250 N $(NH_4)_2C_2O_4$ simultaneously from burets into boiling water. Crystals formed immediately. The mother liquor was removed by suction, the crystals washed repeatedly with water, and dried.

For the solubility experiments, the calcium oxalate and urea were weighed into 100 cc. volumetric flasks and water added to volume with adjustment of temperature. The solutions were thoroughly mixed, transferred to Florence flasks and shaken over night in a mechanical shaker. The calcium oxalate was determined by a modification of the method of Fiske and Adams.[3] The solutions were filtered through a fine ash-free filter paper and 25 cc. of the filtrates transferred to platinum crucibles, dried on a hot plate and heated in a flame to dull redness for 15 minutes. The ash was dissolved in an excess of 0.02 N HCl and titrated back with 0.02 N NaOH from a calibrated micro-buret using methyl red as an indicator.

TABLE I.

Solubility of calcium oxalate in aqueous solutions of urea (100 mg. of calcium oxalate, $CaC_2O_4.H_2O$ + urea and made up to 100 cc. with CO_2-free water). Temp. 22.6°. The solubility of calcium oxalate is expressed in mg. per 100 cc. of solution.

Urea (gm.)	—	0.06	0.15	0.80	3.00	16.00	50.00
Solubility $CaC_2O_4.H_2O$	0.63	0.66	0.70	0.82	1.28	2.80	8.02

For the uric acid solubility experiments uric acid was purified according to the method of Folin.[4] The experiments were set up as follows:

The desired amounts of uric acid and urea were weighed into volumetric flasks and water added to volume. The solutions were mixed, transferred to Florence flasks and shaken in a mechanical shaker for 1 hour. At the end of this time, the flasks were removed and the solutions allowed to stand for 1 hour for the undissolved uric acid to settle. By this time all the solutions were usually clear except those containing about 10% urea which remained opalescent. Samples of each were transferred to centrifuge tubes and centri-

[3] Fiske, C. H., and Adams, E. T., *Am. Chem. Soc.*, 1931, **53**, 2498.

[4] Folin, O., *J. Biol. Chem.*, 1922, **54**, 153.

fuged until clear, precautions being taken to prevent evaporation. The amounts of uric acid in solution in the various samples were determined by Folin's[5] method for the determination of uric acid in blood.

TABLE II.

Solubility of uric acid in solutions of urea. Grams of urea + 80 mg. uric acid made up to 100 cc. with CO_2-free water (with adjustment temperature). The solubility of uric acid is expressed in mg. per 100 cc. of solution. Temp., 26.2°.

Urea	Solubility uric acid	Urea	Solubility uric acid	Urea	Solubility uric acid
gm.	mg.	gm.	mg.	gm.	mg.
—	4.39	6.00	9.21	16.00	8.59
0.16	4.78	8.00	9.73	18.00	7.80
0.40	5.33	10.00	11.52	20.00	7.68
2.00	6.44	12.00	10.67	—	—
4.00	6.83	14.00	9.30	—	—

Sodium urate was prepared by adding sodium acetate to a saturated solution of lithium urate, the precipitate was washed repeatedly in water, filtered off and dried. 200 mg. were weighed into each of two 100 cc. volumetric flasks, 3.00 gm. urea added to one, and each made up to volume at 34°. The 2 solutions were subsequently treated as in the previous experiment and the uric acid in solution determined by the same method. Temperature, 34°.

Solubility of sodium urate in water (mg. per 100 cc. sol.) 142.

Solubility of sodium urate in 3% urea (mg. per 100 cc. sol.) 180.

In the experiments above, urea increases the solubility of calcium oxalate, uric acid and sodium urate. The solubility of the oxalate rises continuously, there being no indication of formation of molecular compounds. The solubility of uric acid increases with increasing concentrations of urea until 10% urea is reached after which there is a slight decrease in solubility—a solubility curve which may be explained as the resultant of 2 opposing forces (a) the peptizing action of urea by which it tends to increase the solubility of uric acid, and (b) some reaction between urea and water by which water is made less available as a solvent. The latter effect would predominate in high concentrations of urea.

The peptizing action of urea may therefore be considered an important factor in maintaining these compounds in solution in urine. When 3% urea is present, 1500 cc. of solution would contain 19.2 mg. calcium oxalate, an amount approximating the average daily output of a normal individual.

1500 cc. of a solution containing 3% urea would dissolve about

[5] Folin, O., *J. Biol. Chem.*, 1930, **86**, 179.

100 mg. of uric acid, while the normal daily output lies at 0.25 gm. or above. Urea, therefore, could not maintain this amount of the free acid in solution. 1500 cc. of 3% urea could dissolve 2.1 gm. of sodium urate, an amount well above the daily output of a normal individual. Ammonium urate has about one-half the solubility of sodium urate, and therefore on a concentrated urine with high uric acid content the limit of saturation would be surpassed. Uric acid is present wholly in the form of the free acid only at a pH of 5 or less and is present wholly as the urate only at a pH of about 8. Hence in most normal urines mixtures of free acid and salt occur.

Conclusions. The peptizing action of urea may be an important factor in increasing the solubility of these sparingly soluble compounds which are normally present in urine at about their saturation limit.

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A Natural Infection of the Sharp-tailed Grouse and the Ruffed Grouse by Pasteurella tularensis.

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The widespread destruction of rabbits and grouse in Minnesota during 1925 and 1926 led to a consideration of tularemia in epizootic proportions as a cause of the periodic decimation of wild rabbits and possibly of grouse. Green and Wade[1] reported that ruffed grouse were susceptible to experimental infection with *Pasteurella tularensis.* Parker and Spencer[2] had previously considered tularemia as a possible disease of grouse and had produced a fatal infection in a blue grouse. Tularemia as a natural disease of birds was definitely established by Green and Wade,[3] when they isolated *Pasteurella tularensis* from a quail dying in the wild. Parker,

[1] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **15**, 515.

[2] Parker, R. R., and Spencer, R. R., *Sixth Biennial Report of the Montana State Board of Entomology*, 1925-1926, 30.

[3] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 626.