The present synthesis is believed to be more satisfactory than those previously reported for aspartic acid.

Phthalimido malonic ester, prepared from potassium phthalimide and bromodiethylmalonate, was converted to sodium phthalimido malonic ester by allowing it to react with molten metallic sodium suspended in toluene. A stable addition product was then formed as a dark colored oil by the reaction of chloroacetic ester with sodium phthalimido malonic ester. This product was readily hydrolyzed by hydrochloric acid with the formation of d,l aspartic acid, phthalic acid, carbon dioxide, and ethyl alcohol. Side reaction products are not probable.

The acid hydrolysate was evaporated to precipitate the phthalic acid which was removed by filtration. Hydrochloric acid was removed by the addition of the calculated quantity of silver oxide to the boiling diluted filtrate. After removing the precipitate of silver chloride and evaporating the filtrate to a small volume d,l aspartic acid crystals separated after standing for 3 days. Upon recrystallization from hot, 50% ethyl alcohol these crystals darkened and decomposed over the range, 311-325°C. Within the limits of experimental error they were shown to contain the theoretical quantity of amino nitrogen.

Additional experiments on the reaction of ethylene bromide, ethylene chloride, methylene bromide, beta chloropropionitrile, beta chloropropionic ester, and trimethylene bromide were carried out. In all cases the liberation of halide ions was nearly complete, but only with trimethylene bromide was the expected addition product formed. This reaction has been used by Sorenson[10] for the synthesis of proline.

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Use of Phenol Red in the Addis Test of Renal Function.

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Under certain special conditions the rate of urea excretion in the urine is directly proportional to the blood urea concentration so that the ratio $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ becomes a constant.[1] Under these

[10] Sorenson, *Z. physiol. Chem.*, 1908, lvi, 236.

[1] Addis, T., and Drury, D. R., *J. Biol. Chem.*, 1923, lv, 105.

conditions this ratio varies only with the amount of functioning renal tissue[2, 3] and can be used as an accurate method for measuring renal function in man.[4] It has recently been shown[5] in the

TABLE I.

Time of Urine Collection	Urine volume cc./hr.	Excretion		Blood sample	Plasma Concentration		Urine rate / Plasma concentration	
		Phenol red mg./hr.	Urea mg./hr.		Phenol red mg./100cc.	Urea mg./100cc.	Ratio: Phenol red	Urea
11:00-11:20	620	858	1737	11:10	5.80	36.2	149	48
11:20-11:40	300	599	1460	11:30	3.90	28.5	153	51
11:40-12:00	750	360	1366	11:50	3.25	26.2	160	52

[2] Taylor, F. B., Drury, D. R., and Addis, T., *Am. J. Physiol.*, 1923, lxv, 55.

[3] Addis, T., Myers, B. A., and Oliver, J., *Arch. Int. Med.*, 1924, xxxiv, 243.

[4] Addis, T., *Arch. Int. Med.*, 1922, xxx, 378.

[5] MacKay, E. M., and Oliver, J., *J. Exp. Med.*, 1930, li, 161.

rabbit that phenol red is excreted in a manner similar to urea in so far as the relation of the urine rate to the plasma concentration is concerned. This has led us to examine the possibility of using phenol red in place of urea in performing the Addis test of renal function[4] which has been referred to. The results presented in Table I which were obtained from a subject without renal disease after the injection of 1 gm. phenol red* during an urea diuresis indicate that it may be feasible to use phenol red in place of urea to determine the Addis excretory ratio. The difference between the urea and phenol red ratios which is apparently constant will be discussed elsewhere. There are certain advantages over urea in the use of the dye, particularly in the determinations in the plasma and urine. The actual concentrations need not be measured. After appropriate dilution and alkalinization the serum and plasma are compared in the 2 cups of a good colorimeter and the ratio calculated from the readings and respective dilutions. The clinical application of the use of phenol red in this manner is being investigated.

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Inactivation of Staphylococcus Bacteriophage by Toluidine Blue.

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The inactivation of staphylococcus bacteriophage by methylene blue was reported by Schultz and Krueger.[1] Further tests in this laboratory with various samples of methylene blue* have given practically the same results, concentrations of 0.05% of the dye inactivating the bacteriophage within 24 hours. In higher dilutions longer periods of time were required for inactivation, whether the tests were carried out at room temperature or at 37°C. In only a few isolated cases was an inactivation obtained with concentra-

* We are indebted to the firm of Hynson, Westcott and Dunning of Baltimore for making this study possible through their very generous cooperation in supplying strong phenol red solutions suitable for intravenous use.

1 Schultz, E. W., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 101.

* Merck's U. S. P. Methylene Blue (3 samples); Coleman and Bell, U. S. P. Methylene Blue; National U. S. P. Methylene Blue (2 samples); Grubler's Methylene Blue.