between the specific heats of the transition complex and the reactants will be mainly due to any changes of solvation that occur on forming the transition complex, and these depend largely on the change of charge, which is here zero. Moreover, these changes of solvation would also be reflected in the entropy of activation, and so in an abnormal A-factor ; and for this reaction A is nearly normal.

This work will be reported in full elsewhere.

E. F. Caldin
G. Long

University of Leeds.

F. W. Trowse

University of Manchester.

Jan. 13.

[1] Bell, R. P., "Acid-Base Catalysis", chapter 8 (Oxford, 1941).
[2] cf. Chance, *J. Franklin Inst.*, **229**, 455, 613, 737 (1940).

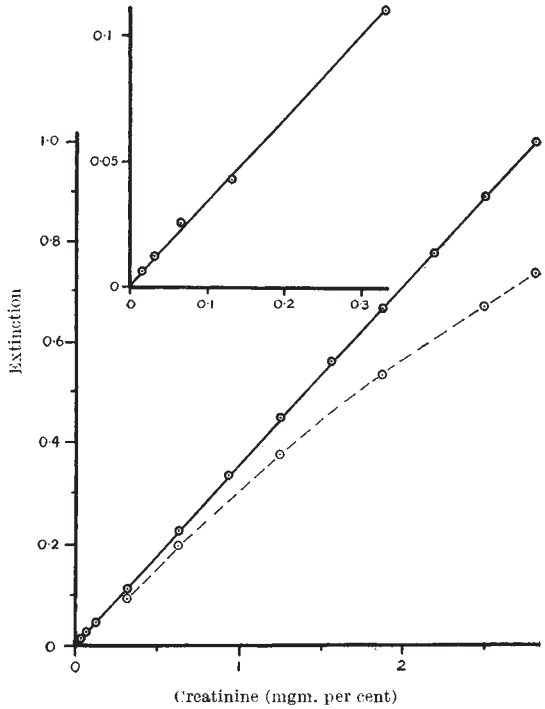
Determination of Creatinine by the Jaffé Reaction

It has often been stated that the colour-producing reaction between creatinine and alkaline picrate does not obey Beer's law[1–3]. This implies that a calibration graph relating creatinine concentration to light extinction is not a straight line. Curves have been obtained with photoelectric absorptiometers using filters which pass an appreciable band of wave-lengths, for example, 495–550 mμ [4].

Beer's law, however, is only strictly true for monochromatic light[5]. It does not appear to be generally known that an instrument measuring on a very narrow band of wave-lengths gives a linear calibration graph for creatinine, although Borsook and Dubnoff[6] mentioned this fact in describing their method for estimating creatine. The accompanying graph was plotted from readings obtained with a Beckman spectrophotometer for a series of dilutions of a standard solution of creatinine zinc chloride. The modification of the Jaffé reaction published by Bonsnes and Taussky[1] was employed, but the linearity is not peculiar to this particular method. Within the limits of probable volumetric and reading errors, the points fall on a straight line over the range of concentrations which can be read satisfactorily.

The wave-length 520 mμ seems to be the most convenient to use. At longer wave-lengths the sensitivity decreases. At shorter wave-lengths the sensitivity is greater ; but this is not an advantage, because at 520 mμ it is already sufficient for the accuracy of the method as a whole. Although the anomalies in light absorption at low concentrations of creatinine reported by Garner[3] suggest that below 515 mμ the relationship would not be linear, similar graphs were in fact obtained at 490 mμ and at 480 mμ, the range of concentrations which could be read being about half as great as at 520 mμ. There was no evidence of any systematic departure from linearity, but apparently fortuitous deviations at low concentrations (see inset graph) became relatively greater, or appeared for the first time, as the wave-length was reduced. The slit-width had to be increased to work at the shorter wave-lengths.

The calibration graph is reproducible if the conditions are kept constant, but this may be difficult to achieve. It has been found that the time for which the colour is allowed to develop before reading is not critical within the limits given by Bonsnes and Taussky, but the temperature of the solutions at the time of reading does have an appreciable effect.



——— Calibration graph for standard solutions of creatinine zinc chloride read with the Beckman spectrophotometer. λ = 520 mμ ; slit-width, 0·04 mm. Inset : lower part of the graph drawn on expanded scales. Concentrations refer to creatinine base in the solutions, after diluting but before adding alkaline picrate. The lowest point corresponds to a creatinine concentration about a tenth of that found in a normal serum filtrate. — — —, Calibration curve for the same solutions read with a 'Spekker' absorptiometer, with Ilford 604 filters (nominal band-width 500–540 mμ)

Rather than standardize these and other variables, it is probably simpler to read one or more known solutions with each batch of unknowns, the linearity of the graph then enabling it to be drawn for the conditions prevailing at the time. If the temperature of the solutions alters through heating up while in the instrument, it is advisable either to adopt a standard reading procedure or to warm all the solutions to a suitable temperature beforehand.

Three other practical points may be worth mentioning. Acetone is one of the many substances which give the Jaffé reaction, and it should not be used for drying glassware[7]. Standard creatinine zinc chloride solutions in $N/10$ hydrochloric acid will not keep for more than a few weeks at room temperature[8] : they can be kept under toluene in the refrigerator. Finally, the reagent proportions suggested by Bonsnes and Taussky have the advantage that the alkaline picrate solution does not form a precipitate, and will keep for longer in the mixed form.

G. R. Hervey

Medical Research Council
Department of Experimental Medicine,
Cambridge.

Dec. 18.

[1] Bonsnes, R. W., and Taussky, H. H., *J. Biol. Chem.*, **158**, 581 (1945).
[2] Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry", 472 (Philadelphia : Blakiston, 1947).
[3] Garner, R. J., *Nature*, **170**, 460 (1952).
[4] Peters, J. H., *J. Biol. Chem.*, **146**, 179 (1942).
[5] Gibb, T. R. P., "Optical Methods of Chemical Analysis", 74 (New York : McGraw-Hill, 1942).
[6] Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **132**, 559 (1940).
[7] Jaffé, M., *Z. physiol. Chem.*, **10**, 391 (1886).
[8] Borsook, H., *J. Biol. Chem.*, **110**, 481 (1935).