CXXXIII. A NOTE ON THE ESTIMATION OF ADRENALINE.

By LESLIE CHARLES BAKER and GUY FREDERIC MARRIAN.

From the Department of Physiology and Biochemistry, University College, London.

(*Received June 30th, 1927.*)

Introduction.

During the progress of other work in this laboratory it was necessary to carry out quantitative estimations of the adrenaline present in the small adrenal glands of the rat and pigeon. The well-known colorimetric method of Folin, Cannon and Denis [1913], described for estimations on glands of about 2 g. in weight, seemed the most suitable for this purpose.

On adapting this method to the small glands of about 0·03 g. weight that were being used, results were obtained that showed considerably wider variation than was anticipated. At the same time it was noticed that, under the conditions of our adaptation, the rate of colour change of the adrenaline solution differed from that of the uric acid standard solution.

Accordingly it was decided to investigate the effect of such variable factors as temperature on the rate of colour production of solutions of adrenaline, using the glasses of a Lovibond tintometer as a standard against which to match the blue colour.

The possibility of loss of adrenaline due to oxidation in the method of extraction employed by Folin, Cannon and Denis was also examined and found to be small but definite. This loss takes place after the addition of sodium acetate to the hot hydrochloric acid extract and was avoided by using cold trichloroacetic acid as the extracting agent. This acid proved satisfactory in every way and gave a clear protein-free filtrate.

Apparatus.

The best source of light for use with a tintometer is daylight from a north window. However, as we wished to work at any time and during the winter months an artificial source of light was necessary. A stout cardboard box was painted black inside and placed upon its side, which was 42 cm. long. Two daylight lamps were inserted in the end, one above the other and 15 cm. apart, and across the other open end of the box two sheets of the special transparent paper supplied by the Tintometer Company, Salisbury, were placed close together. A third sheet of this paper was inserted about 18 cm. nearer the lamps

than the other two sheets. Such an arrangement gave a fairly large and evenly illuminated field and the tintometer was placed at such a distance that the structure of the transparent paper could not be seen. Two small weighing bottles, of internal diameter 2·35 cm., served as cells for holding the solutions to be matched. During the day both daylight lamps were employed but at night it was found easier to match if only one was used. A thermostat was maintained at 20° (or the appropriate working temperature) and held the matching cells and all the reagents and vessels used in producing the blue colour.

Temperature and other factors.

By using a tintometer the blue colour may be matched against a non-variable standard and the difficulty of matching two colours of not quite the same tint, such as those given by adrenaline and uric acid with the phosphotungstic "uric acid reagent," is avoided.

In order to plot time-intensity curves of the blue colour developed, an accurately weighed amount of adrenaline, between 0·03 and 0·04 g., was dissolved in 10 % trichloroacetic acid and the volume made up to 100 cc. with this acid. Small portions of this standard solution, between 1 cc. and 5 cc., were diluted to 25 cc. with 10 % trichloroacetic acid and 3 cc. of such a solution were run into a 25 cc. flask, diluted somewhat with water and placed in the thermostat. In this way the concentration of adrenaline could be readily varied while the acid concentration remained constant. When sufficient time had been allowed for the flask and its contents to reach the appropriate working temperature, 0·5 cc. of the phosphotungstic "uric acid reagent" described by Folin and Trimble [1924] was added, washed in with a little distilled water, the time noted, 5 cc. of a saturated solution of sodium carbonate added, washed in and made up to a final volume of 25 cc. with distilled water. The flask was then shaken briskly and a suitable amount of the solution poured into the matching cell. The blue colour was matched at measured intervals of time after the addition of the sodium carbonate. The amount of adrenaline estimated in these experiments was of the order of that present in 0·03 g. of adrenal gland. It was found that the blue glasses of the tintometer required the admixture of about 10 % of the yellow glasses in order to match the tint produced by adrenaline, but only the value of the blue glasses was recorded. It was difficult to match accurately any solution paler than five or six blue units.

The time-intensity curves for different concentrations of adrenaline were plotted at 20°. These curves, shown in Fig. 1, rise to a maximum in almost exactly 20 minutes and demonstrate clearly that, over the range studied, the concentration of adrenaline does not affect the time of maximum colour development at any one temperature.

The rate of colour development for one concentration of adrenaline at 14°, 16° and 25° was next studied. Fig. 2 shows the curves obtained; clearly, the reaction velocity is greater at higher temperatures, the time taken to reach a maximum increasing as the temperature falls; at 25° the maximum is reached

after 10 minutes, at 16° after about 35 minutes and at 14° after 45 minutes. The curves at 25°, 16° and 14° are typical curves selected from a number obtained at those temperatures, while the curve at 20° is an average of all those obtained for that concentration at that temperature.

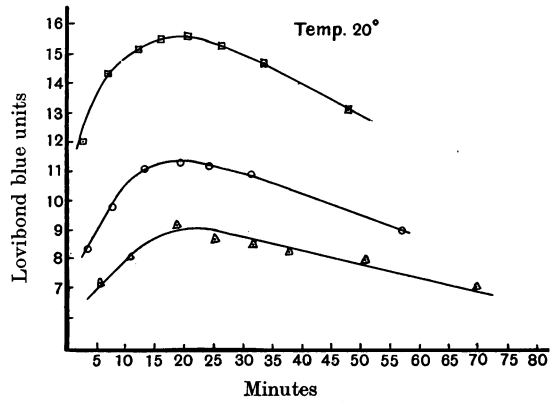

Fig. 1.

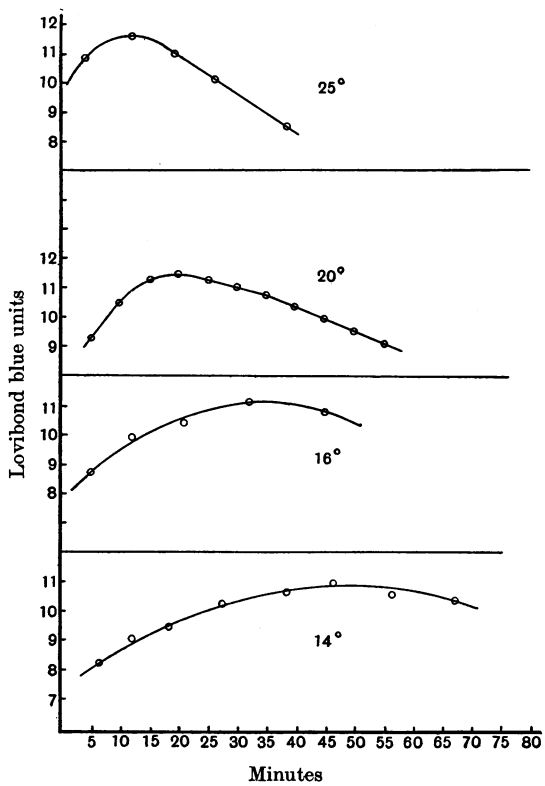

Fig. 2.

In order to estimate adrenaline in solutions of unknown strength, a calibration curve was constructed. Such a curve is of course dependent on the size of cell for matching used and possibly also on the particular specimen of "uric acid reagent" employed and must therefore be independently constructed by each worker. As a working temperature 20°, at which maximum colour develops in 20 minutes, was selected as being the most convenient. The known adrenaline content was plotted against the blue colour developed after 20 minutes. Three such points were taken from the curves in Fig. 1 and two more were obtained from separate determinations, one for a higher and the other for a lower concentration of adrenaline. The calibration curve is a mean straight line drawn through these points. Other known concentrations of adrenaline were then estimated and marked on the graph. From these the possible maximum experimental error was calculated to be 7 %.

Oxidation.

The possibility of loss of adrenaline by oxidation during the boiling in hydrochloric acid solution after addition of sodium acetate, in the method of Folin, Cannon and Denis, was investigated with solutions of pure adrenaline and, except where mentioned below, following their technique exactly. In each experiment about 0·008 g. of adrenaline was used, as this was a fair average of the amount estimated by these workers in 2 g. of adrenal gland. After addition of sodium acetate, in the first experiment the solution was brought to the boil, made up to 100 cc., roughly divided, one half cooled rapidly under the tap and the other half allowed to cool slowly on the bench. 1·25 cc. of one half was then pipetted into a 25 cc. flask and matched in the way described in the last section. 1·25 cc. of the other half was similarly estimated. In the second experiment boiling after addition of sodium acetate was continued for one minute and in the third experiment for two minutes; the subsequent procedure in these two experiments was the same as in the first.

Table I shows that no significant loss takes place if the method of Folin, Cannon and Denis be followed exactly, but that if the boiling be continued for 2 minutes and the solution allowed to cool slowly there is a 10 % loss. Thus when working with quantities of adrenaline a hundred times as small, appreciable loss might readily occur.

Table I.

Exp.	Treatment after addition of NaAc	Units of blue with cooling: Rapid	Units of blue with cooling: Slow	Units of blue in control	Loss: Rapid	Loss: Slow	% loss: Rapid	% loss: Slow
1	Brought to boil	12·4	12·0	12·6	0·2	0·6	2·0	5·0
2	Boiled for 1 min.	12·1	11·6	12·6	0·5	1·0	4·0	8·0
3	Boiled for 2 mins.	11·7	11·4	12·6	0·9	1·2	7·5	10·0

Extraction of adrenal glands.

The efficiency of a 10 % aqueous solution of trichloroacetic acid as an extracting agent was investigated. A number of fresh rat adrenals were available.

These were minced finely with scissors to give a fairly homogeneous mash and three samples were weighed out, two of about 0·03 g. and one of 0·06 g. The adrenaline content of one 0·03 g. sample was estimated directly and to each of the other two samples a volume of an adrenaline solution equal to that of a previously estimated volume from the same solution was added. The glands were then ground fine and the adrenaline content of each estimated. The results are set out in Table II. The very slight loss observed shows that trichloroacetic acid is a very suitable extracting agent.

Table II.

Exp.	Weight of gland in g.	Units of blue observed	Control blue units	Blue units adren. pres. calc. from 0·0315 g.	Units of blue calc., *i.e.* control + calc.	Loss blue units	% loss
1	0·0315	4·9	5	—	—	—	—
2	0·0329	9·9	5	5·1	10·1	0·2	2·0
3	0·0629	14·5	5	9·8	14·8	0·3	2·0

Our extracts were accordingly made in the following way. The adrenal glands, in which the adrenaline was to be estimated, were carefully freed from connective tissue and transferred to a tared pyrex test-tube cut down to half size; this was weighed again. A little purified sand and a few drops of about 10 % trichloroacetic acid solution were added and the gland was finely ground in the tube with a glass rod, 3 cc. of the 10 % trichloroacetic acid were added and the sand and precipitated protein agitated, the sediment was allowed to settle and the liquid filtered through a wad of cotton-wool into a 25 cc. flask. The residue in the tube was washed first with 5 cc. and then with 3 cc. of distilled water plus a few drops of trichloroacetic acid solution, the washings were filtered into the flask and the latter set in the thermostat. The extract was then made up and matched in the manner described in an earlier section. Table III shows some results for a number of normal male rats.

Table III. *Starved* 6–8 *hours before experiment.*

Rat	Body weight (g.)	Rect. temp. ° C.	Weight adrenal glands (g.)	per 100 g. body weight (g.)	mg. adrenaline	Adrenaline mg. per 1 g. gland	Adrenaline mg. per 100 g. body weight
1	126	37·7	0·0386	0·0306	0·086	2·23	0·068
2	130	38·1	0·0334	0·0257	0·083	2·49	0·064
3	119	38·2	0·0338	0·0284	0·074	2·19	0·062
4	124	36·6	0·0376	0·0303	0·071	1·89	0·057
5	129	37·3	0·0320	0·0248	0·058	1·83	0·045
Average	126	37·6	0·0337	0·0267	0·074	2·13	0·059

The Authors wish to express their thanks to Prof. J. C. Drummond for much helpful advice and his constant interest in the work. One of them (G. F. M.) is indebted to the Medical Research Council for a personal grant.

REFERENCES.

Folin, Cannon and Denis (1913). *J. Biol. Chem.* **13**, 477.
Folin and Trimble (1924). *J. Biol. Chem.* **60**, 472.