the quantity of protein present in the original macro method of Malloy and Evelyn. This violet colour led us to try an alkaline solution of phenolphthalein buffered to pH 10 as an artificial standard. This solution has a maximum absorption between 560 m μ and 500 m μ with a peak at 540 m μ and was found to be stable at room temperature for a period of at least three months.

Summary

A rapid, simple and accurate method for the estimation of bilirubin in small quantities (0.1 ml. or less) of serum or plasma is described. The method is suitable for use in laboratories equipped with standard photoelectric instruments and has proved valuable, particularly in following the progress of erythroblastotic infants.

Thanks are due to Dr. E. M. Darmady for his help and encouragement.

REFERENCES

- Dangerfield, W. G., and Finlayson, R. (1953). *Journal of Clinical Pathology*, **6**, 173.
 Hsia, D. Y., Hsia, H., and Gellis, S. S. (1952). *J. Lab. clin. Med.*, **40**, 610.
 Jendrassik, L., and Gróf, P. (1938). *Biochem. Z.*, **297**, 81.
 King, E. J., and Coxon, R. V. (1950). *Journal of Clinical Pathology*, **3**, 248.
 Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.*, **119**, 481.
 Patterson, J., Swale, J., and Maggs, C. (1952). *Biochem. J.*, **52**, 100.
 Powell, W. N. (1944). *Amer. J. clin. Path.*, **14**, Tech. Sect., **8**, 55.
 With, T. K. (1943). *Hoppe-Seyl. Z. physiol. Chem.*, **278**, 120.

Transistor-Amplifier Units for Absorptiometry

P. W. PERRYMAN AND D. H. RICHARDS

From the Group Biochemistry Department, Southend-on-Sea Hospital, Rochford, Essex

(RECEIVED FOR PUBLICATION AUGUST 11, 1955)

On account of the property of the junction-transistor to amplify small currents in low impedance networks this device is particularly well suited to amplify the output of selenium photo-cells. The production and use of two simple transistor units designed to increase the sensitivity of selenium-cell absorptiometers are described below.

In the commoner types of absorptiometer such as are widely used in hospital laboratories, the output of a selenium cell is coupled directly to a microammeter of some 10 micro-amps full-scale deflection. These instruments, though admirable for many routine measurements, are of insufficient sensitivity for comparison of very small differences of colour density, and for such purposes it is necessary to employ more elaborate and expensive spectrophotometric equipment.

Efforts to improve such elementary absorptiometers may be made by using good narrow-band filters to give the best possible match between the wavelength of the light used and the absorption maximum of each particular test. Such efforts are, however, frequently defeated by the impossibility of then obtaining sufficient photo-cell current to give full scale deflection for "100% transmission" owing to the low transmission of many narrow-band filters.

Even higher sensitivity may be required to permit the use of "interference filters" of still narrower band width. Since these filters are now available having any specified pass-band in the visible range to suit the absorption characteristics of any particular system of analytical interest, they offer striking possibilities of greatly improved sensitivity and selectivity of absorptiometric analyses providing adequate photo-cell sensitivity is available.

Type I Amplifier

The type I amplifier is shown in Fig. 1. The output leads from the selenium cell of a conventional photoelectric colorimeter are connected as shown, the negative lead to the "base" of a Mullard O.C.71 junction-transistor and the positive lead to the "emitter."

The negative terminal of the colorimeter galvanometer is also connected to the emitter and the positive terminal of the galvanometer to the positive terminal of a single 1.5-volt miniature dry-cell. This latter is in series with 300-ohm resistance, and finally the circuit is completed by connecting this to the "collector" of the transistor.

FIG. 1

As in the condition of no-illumination there will be a small "dark current" registering on the galvanometer; the shunt network connected as shown in Fig. 1 is used to supply a small adjustable counter-current to the galvanometer so that zero may be set by manipulation of the 0.5-megohm potentiometer.

The whole unit may be mounted in a small perspex museum box, 2 in. $\times$ 3 in. $\times$ 3 in., as shown in the photograph.

In order that the colorimeter may continue to be used in the normal manner when required, it is convenient to bore a $\frac{1}{2}$ -in. hole on one side of the casing and fit a miniature 4-pin radio socket. Galvanometer and photo-cell leads are soldered to these sockets, and insertion of a suitably wired plug gives the conventional colorimeter connexions. The appropriate leads from the amplifier unit are wired to a second plug and this is inserted when amplification is required. To use the amplified colorimeter the light shutter is first closed and the light source switched on, the appropriate filter is put in and the amplifier unit connected. With no illumination and the blank

solution in the absorption cell, the galvanometer needle is adjusted to ∞ (100% absorption) position by manipulation of the potentiometer knob. The colorimeter shutter is then carefully opened until the galvanometer pointer reads zero (100% transmission). Absorption readings of test solutions can then be read off in the usual way.

The amplifier unit gives remarkable stability and the drain on the 1.5-volt dry cell is very small indeed.

With this simple device it has been found that the sensitivity of an ordinary photo-electric colorimeter such as the E.E.L. portable may be increased up to the limits of the mechanical and optical systems of the instrument. The current amplification of the type 1 transistor unit is some 30 times and allows the use of narrow-band filters, such as the Ilford spectrum range, over the whole visible region. On the un-amplified instrument many of these filters pass insufficient light to permit full-scale deflection, particularly in tests where the colour of the blank solution is considerable. An example is given in Fig. 2, which shows the effect of type 1 amplifier when used for the alkaline picrate method of blood creatinine estimation. The Chance O.B.2 filter is the best choice with the normal instruments; the deep yellow picrate reagent blank cuts off too much light to allow the use of the sharper cut O.B.1 or Ilford spectrum blue green. The resulting line A (Fig. 2) is too flat to be of much use; coupling in the amplifier, however, permits the use of the narrow band Ilford spectrum blue-green filter and gives a much better slope (line B, Fig. 2).

FIG. 2.—B, with Type I amplifier Ilford spectrum blue-green.
A, normal instrument filter Chance OB2.

The Type II Amplifier

In the course of further experiments it has been found that certain advantages can be reaped by applying a fraction of the output of a transistor amplifier back to the photo-cell in a reverse direction to the normal photo-current output. This is illustrated in Fig. 3. A small reverse current is applied to the photo-cell by the potentiometric network energized by a 1.5-volt miniature dry-cell through the 0.5-megohm potentiometer, part of which, say 5K, may be an additional series variable resistance to give "fine" and "coarse" adjustment.

The same network provides the neutralization of dark current in the galvanometer. With this arrangement, when the illumination of the photo-cell is increased, no effect is produced until the photo-current generated is larger than the reverse current referred to above. Further increase in light thereafter produces a current in the galvanometer of from three to four times that of the unamplified photo-cell output. This arrangement deliberately distorts the normal illumination-current output relationship so as to yield increased sensitivity over a restricted range of the galvanometer scale. In practice, the extinction-concentration curve for given solution, filter, and cell combination is

FIG. 3

markedly steeper than that obtained with the unmodified absorptometer; at the same time, there is full control over zero setting, and narrow-band filters can be employed over the visible range with the exception of the extreme blue end.

Fig. 4 shows an example of the use of the arrangement relating to the Zimmerman reaction for esti-

FIG. 4.—C, Ilford spectrum green with amplifier. B, Chance OGI with amplifier
A, Chance OGI. No amplifier.

FIG. 5.—B. Using Type II amplifier. A. Normal instrument.

Position (a). Linear (Circuit 1 type) amplification.

Position (b). A small reverse current applied to the photo-cell gives circuit II type amplification.

Position (c). As (b) but giving approximately double the sensitivity.

mation of 17-ketosteroids. The line A, of rather poor sensitivity, is obtained with the normal instrument; using the same filter with Circuit II amplifier connected the line B, having much improved sensitivity, is obtained; however, Type II also permits the use of a narrow band filter Ilford spectrum green, which gives line C, showing even better sensitivity. It must be emphasized, however, that the high sensitivity obtainable with Type II is produced by electrical distortion of the normal output, and in all cases the extinction-concentration relationship is linear for only two-thirds of the galvanometer scale. This is illustrated in Fig. 5.

The actual degree of linearity will be determined by the circuit component resistances, of which the galvanometer and photo-cell form integral parts. In this work we have applied the arrangement very successfully to the E.E.L. portable colorimeter and to the Mark 1 Hilger-biochem. absorptiometer: with other instruments some amount of experimentation may be needed to arrive at conditions giving linearity over the useful part of the scale. Provided these inherent limitations are recognized, the increased sensitivity makes possible many useful analytical applications.

The performance of a transistor is affected by temperature change, but in practice this factor is not significant, as the time taken for ordinary measurements is quite short and the temperature variations in the normal laboratory over that time are quite insignificant.

Where very high sensitivity is required a two-transistor unit, arranged to give three combinations, has been developed recently.

FIG. 6.—Circuit III. Amplifier unit.