REFERENCES

- Adler, E., Euler, H. v., Günther, G. & Plass, M. (1939). *Biochem. J.* **33**, 1028.
- Borsook, H. & Dubnoff, J. W. (1941). *J. biol. Chem.* **141**, 717.
- Cohen, P. P. & Hayano, M. (1946). *J. biol. Chem.* **166**, 251.
- Dewan, J. G. (1938). *Biochem. J.* **32**, 1378.
- Edson, N. L. (1935). *Biochem. J.* **29**, 2082.
- Euler, H. v., Adler, E., Günther, G. & Das, N. B. (1938). *Hoppe-Seyl. Z.* **254**, 61.
- Fahrlander, H., Favarger, F., Nielsen, H. & Leuthardt, F. (1947). *Helv. physiol. pharmacol. Acta*, **5**, 202.
- Gale, E. F. (1945). *Biochem. J.* **39**, 46.
- Krebs, H. A. (1935). *Biochem. J.* **29**, 1620.
- Krebs, H. A. (1942). *Biochem. J.* **36**, 758.
- Krebs, H. A. & Cohen, P. P. (1939). *Biochem. J.* **33**, 1895.
- Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
- Krebs, H. A. & Eggleston, L. V. (1945). *Biochem. J.* **39**, 408.
- Krebs, H. A. & Eggleston, L. V. (1948a). *Biochem. J.* **42**, 17.
- Krebs, H. A. & Eggleston, L. V. (1948b). *Biochem. Biophys. Acta*. In the Press.
- Krebs, H. A., Eggleston, L. V., Kleinzeller, A. & Smyth, D. H. (1940). *Biochem. J.* **34**, 1234.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
- Ratner, S. (1947). *J. biol. Chem.* **170**, 761.
- Van Slyke, D. D. (1929). *J. biol. Chem.* **83**, 425.

The Determination of Quinine Degradation Product in Blood, and its Absorption in the Chick

BY P. B. MARSHALL AND E. W. ROGERS

The Wellcome Laboratories of Tropical Medicine, Euston Road, London

(Received 27 February 1948)

In a previous publication (Marshall, 1945a) we reported that quinine degradation product (QDP) showed no antimalarial action against *Plasmodium gallinaceum* in young chicks, when given orally in amounts equivalent to an active dose of quinine. Subsequently, Kelsey, Oldham, Cantrell & Geiling (1946) reported that, when given intravenously, the compound showed one third to one fifth the activity of quinine against chick malaria, and less than one twentieth that of quinine against *P. lophurae* when given orally to ducks. Pointing out that QDP was less toxic than quinine, thus making its therapeutic index nearly equal to that of quinine, these workers disagreed with our view that the metabolism of quinine results in an inherently less efficient drug. They suggested that the reduction of both antimalarial activity and toxicity might be due to a decreased rate of absorption in the host.

In order to test this theory, we decided to compare the concentrations of quinine and QDP attained in the red cells of chicks following oral doses of the compounds, and this necessitated the designing of a method of determination of small quantities of QDP. The fluorescence of QDP in ultraviolet light was too feeble for the determination of very small amounts, though it has been used to determine large concentrations (Knox, 1946). We found, however, that the bromothymol blue method (Marshall & Rogers, 1945) was almost as sensitive with QDP as with quinine.

The degradation product, which is prepared by incubating quinine with rabbit liver suspension, was shown by Mead & Koepfli (1944) to be quinine carbostyryl, i.e. quinine with a hydroxyl group substituted in position 2 of the quinoline ring. This confers phenolic properties on the compound, making it unextractable from sodium hydroxide solution, thus providing a ready method for the separation of QDP from quinine. The degradation product can be extracted from ammoniacal solution (Kelsey, Geiling, Oldham & Dearborn, 1944). We have used the basic principles of this method, but emulsification problems made quantitative extraction difficult. However, by modifying the technique suitably, and carefully controlling the shaking operations, a method giving satisfactory recoveries of small amounts of QDP was produced.

METHODS

Quinine degradation product. This was prepared by incubating quinine with rabbit-liver suspension, and extracting and purifying the compound according to the method of Kelsey *et al.* (1944). The melting point of the final product was 250°.

Chicks and dosing. The chicks used were 7–14 days old, and their body weights ranged between 50 and 80 g. A single dose of 200 mg./kg. of quinine or QDP base was given into the crop by a catheter tube. The bases were dissolved in a minimum amount of HCl. Pairs of birds from the quinine and QDP groups were killed at suitable intervals by placing

them in an atmosphere of CO_2 . The blood from each pair was withdrawn from the heart and mixed in a graduated tube, heparin and citrate being added to prevent clotting. The tubes were centrifuged at about $6600 \times g$ for 15 min., and the volume of plasma and red cells recorded. The concentration of quinine or QDP was determined in samples of both plasma and red cells.

Determination of quinine. Quinine was extracted and determined by the bromothymol blue method, previously described by Marshall & Rogers (1945).

Determination of QDP. Add 1 ml. of plasma or red cells to 5 ml. of distilled water in a 100 ml. glass-stoppered, narrow-necked reagent bottle. Add 1 ml. of 5N-NaOH and digest in an oven at 100° for 30 min. Cool and add 1 ml. (or sufficient to make the digest acid) of 5N- H_2SO_4 , followed by 1 ml. of ammonia (sp.gr. 0.88). Cool and add 25 ml. of chloroform containing 4% ethanol. Stopper and shake the bottle for at least 1 min., but not too violently, or a persistent emulsion will be formed. It is sufficient to keep the two phases well mixed for the period of 1 min. Stand until the two layers are clearly separated, then centrifuge the bottle gently to promote complete separation. Suck off the upper aqueous layer as cleanly as possible at the water pump. Transfer the chloroform layer to a 6 x 1 in. glass-stoppered test tube and rinse out the bottle with 5 ml. of distilled water, adding this to the tube. Mix the two layers by inverting the tube several times, centrifuge, and remove the upper layer as before. Repeat the washing of the chloroform layer with a further 5 ml. of distilled water. Shake the chloroform extract with 2.5 ml. of 0.05N-HCl for at least 0.5 min., and test the upper layer to ensure that it is still acid. Centrifuge, and transfer the acid layer by means of a test pipette to a 10 ml. flask. Repeat the acid extraction with a further 2.5 ml. of acid, and add it to the first extract. Warm the flask on a sand bath to drive off dissolved chloroform, and continue the bromothymol blue coupling procedure as for quinine.

It should be noted that if QDP is being determined in the presence of quinine, the quinine must first be removed by shaking the NaOH digest repeatedly with ether until a little of the ether extract, when shaken with dilute H_2SO_4 , shows no fluorescence in the acid layer when viewed in filtered ultraviolet light.

RESULTS

The recoveries of known amounts of QDP added to chick blood are recorded in Table 1. Considering the additional technical difficulties, these values com-

Table 1. Recoveries of known amounts of quinine degradation product added to chick blood

QDP added ($\mu\text{g.}$)	QDP recovered ($\mu\text{g.}$)	Percentage recovery
5	6.0	120
5	5.1	102
10	12	120
10	9	90
25	27	108
25	23	92
35	35	100
35	35	100
50	48	96
50	47	94

pare favourably with the recoveries of quinine by the bromothymol blue method (Marshall & Rogers, 1945).

The plasma and red-cell concentrations of quinine and QDP up to 7 hr. after dosing are shown graphically in Fig. 1. The red-cell concentration of both compounds was higher than the plasma concentration. The quinine curve was characterized by a sharp peak 1 hr. after dosing, followed by a rapid

Fig. 1. Concentrations of quinine (A) and QDP (B) found in the red blood cells (continuous line) and plasma (broken line) of chicks following oral doses of 200 mg./kg. Each point represents two birds.

decline, which confirms our previous observations on the absorption of quinine (Marshall, 1945b). The red-cell concentration curve for QDP did not show a high initial peak, but the concentration declined less rapidly than with quinine. If the areas enclosed by the respective curves are compared, the QDP concentration over the first 7 hr. exceeds that of quinine in the ratio 1.11 : 1.

DISCUSSION

The results show that, over a period of 7 hr. after oral dosing, the total amounts of quinine and QDP appearing in the red cell are approximately the same. In view of the fact that the concentration curve of the degradation product falls off less rapidly than that of quinine, it may be concluded that, on considerations of red-cell concentration, QDP has the greater opportunity for being actively anti-malarial than quinine. The observed lower anti-malarial activity of QDP cannot, therefore, be

attributed to poor absorption in the host, but must be due to a specifically inferior activity against the malaria parasite itself.

SUMMARY

1. A method for the quantitative determination of quinine degradation product (QDP), sensitive to 5 μ g., is described.

2. The red-cell concentration of QDP in chicks during 7 hr. following oral doses is approximately the same as that of an equal dose of quinine, indicating that the previously observed higher anti-malarial activity of quinine is due to higher specific activity on the parasite, and not to superior absorption in the host.

The authors are indebted to Miss C. Alexander and Mr R. Neville for valuable technical assistance.

REFERENCES

- Kelsey, F. E., Geiling, E. M. K., Oldham, F. K. & Dearborn, E. H. (1944). *J. Pharmacol.* **80**, 391.
Kelsey, F. E., Oldham, F. K., Cantrell, W. & Geiling, E. M. K. (1946). *Nature, Lond.*, **157**, 440.
Knox, W. E. (1946). *J. biol. Chem.* **163**, 699.
Marshall, P. B. (1945a). *Nature, Lond.*, **156**, 505.
Marshall, P. B. (1945b). *J. Pharmacol.* **85**, 299.
Marshall, P. B. & Rogers, E. W. (1945). *Biochem. J.* **39**, 258.
Mead, J. & Koepfli, J. B. (1944). *J. biol. Chem.* **154**, 507.

The Relationship between Losses in Labile Liver Cytoplasm and Urinary Nitrogen Excretion

BY ROSA M. CAMPBELL AND H. W. KOSTERLITZ
Physiology Department, University of Aberdeen

(Received 1 March 1948)

When transferred from an adequate stock diet to a protein-free diet, female rats lose 26% and male rats 36% of their liver cytoplasm in 5 days (Kosterlitz, 1944, 1947; Campbell & Kosterlitz, 1946). It has also been observed in many species that under similar conditions the excretion of urinary N gradually falls until a relatively constant value is reached on the fifth or sixth day (Voit, 1866, 1881; cf. Kosterlitz & Campbell, 1945-6). The excretion of this extra N appears to be closely correlated with the loss of 'labile' liver cytoplasm.

METHODS

Animals. Rats of the hooded strain of the Rowett Research Institute were used. The females were 16-18 weeks and the males 13-14 weeks old. The general management was the same as that described previously (Kosterlitz, 1947).

Diets. The protein-free diets used were the basal diets of Procedures 1 and 3 described previously (Campbell & Kosterlitz, 1948a). Apart from vitamin supplements, 2% agar, 3% salts and 10% lard, they contained either 60% potato starch and 25% sucrose (Exps. 1, 3, 4) or 85% sucrose (Exp. 2). Whole liver concentrate was given in Exps. 1, 3 and 4 but not in Exp. 2, in which the supplements of B vitamins were wholly synthetic (Procedure 3).

Experimental procedure. Urine and faeces were collected by the method of Ackroyd & Hopkins (1916). The flasks for the collection of urine were charged with 5 ml. of 0.2 N-H₂SO₄.

The funnels were washed down twice daily first with 20 and then with 25 ml. of 0.2 N-H₂SO₄, and at the end of each daily period with water. The combined urine and washings were made up to 250 ml. Urinary N was determined by the micro-Kjeldahl method (see Kosterlitz, 1947). In the experiments with sucrose, the whole faeces and, in those with potato starch, suitable samples of the finely ground faeces were ashed by the procedure of Chibnall, Rees & Williams (1943); the period of ashing, however, was reduced to 2 hr. after the clearing of the digestion mixture. A sample containing about 0.2 mg. N was used for distillation.

Calculation of the results. Each experimental period lasted for 7 days. The mean of the daily urinary N excretions on the sixth and seventh days was taken as the basal value. 'Extra N' in urine was the difference between the N actually excreted and the basal N. All values were expressed as mg./100 g. initial body weight. In the regression equations, NNS_t stands for labile non-glycogen non-lipid liver solids (mg./100 g. body weight), N_t for labile protein N (mg./100 g. body weight) and t for days of protein-free diet.

RESULTS

Faecal N and basal urinary N (Table 1). The values for faecal N from the second to the seventh day of the protein-free diet did not differ significantly from day to day within each experiment and were therefore pooled. In the rats which received the protein-free diet with 60% potato starch (Exps. 1, 3, 4), the mean daily faecal N values were high because of the