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 $\mu\text{g.}$, 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.

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

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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- H_2SO_4 .

The funnels were washed down twice daily first with 20 and then with 25 ml. of 0.2 N- H_2SO_4 , 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

Table 1. *Excretion of 'extra N' in the urine, and loss of protein + nucleic acid N from the liver, during the first 5 days of a protein-free diet*

Experimental diet	No. and sex of rats	Initial body wt. (mean and range) (g.)	Mean daily food intake (g. and cal.)	Results/100 g. body wt.				
				Mean daily faecal N (2nd-7th day) $\pm$ s.e. of the mean (mg.)	Mean daily basal urinary N (6th and 7th day) $\pm$ s.e. of the mean (mg.)	Total 'extra N' in urine (1st-5th days) (mg.)	Total loss of protein + nucleic acid N from liver (1st-5th days) (mg.)	Loss of liver N/ 'extra N' in urine
1. Protein-free with starch following stock diet	4 ♀	230 (216-244)	7.2 (24.5)	20.8 $\pm$ 0.7	8.4 $\pm$ 0.5	53	28	0.53
2. Protein-free with sucrose following stock diet	4 ♀	224 (197-262)	6.15 (26.6)	9.0 $\pm$ 0.6	17.1 $\pm$ 1.0	47	28	0.60
3. Protein-free with starch following stock diet	4 ♂	318 (308-338)	7.2 (24.5)	21.3 $\pm$ 0.7	9.6 $\pm$ 0.2	41	39	0.95
4. Protein-free with starch following one week 54% casein diet	4 ♂	271 (248-287)	7.1 (24.2)	24.5 $\pm$ 1.0	11.7 $\pm$ 0.6	91	59	0.65

In Exp. 4, the liver N on the 5th day of a protein-free diet following the stock diet was used for calculation instead of that on the 5th day of a protein-free diet following the 54% casein diet; therefore the calculated N loss may possibly have erred on the large side.

low digestibility of potato starch (Campbell & Kosterlitz, 1948*a*), whilst in the rats receiving the protein-free diet with sucrose (Exp. 2) the faecal N was low. On the other hand, the reverse holds for the basal urinary N values: they were low in the rats receiving potato starch and high in those receiving the sucrose diet. Thus, the presence or absence of potato starch caused only little variation in the total basal (urinary + faecal) N excretion on the last 2 days of the experimental periods; the daily means were 29.2, 26.1, 30.9 and 36.2 mg./100 g. body weight for Exps. 1-4 respectively. The slightly raised value in Exp. 4 can probably be accounted for by the fact that these rats were transferred from a high protein to the protein-free diet.

'Extra N' in urine and loss of protein + nucleic acid N from the liver (Tables 1 and 2). The daily urinary 'extra N' excretions (actually excreted N - basal N) were totalled for the first 5 days of the protein-free diet in order to obtain the total 'extra N' excreted during this period. This value showed only small variations in Exps. 1-3, in which male or female rats were transferred from the stock diet (N = 3.2%) to the protein-free diet. On the other hand, it was considerably raised in Exp. 4 in which the protein-free diet followed a 54% casein diet.

The losses of protein + nucleic acid N, which may be expected to occur from the liver during the same period, were calculated from the protein + nucleic acid N contents of livers, obtained in earlier experiments (Kosterlitz, 1947; Campbell & Kosterlitz, 1948*b*). They are summarized in Table 2. The ratios of 'loss of liver N/extra N in urine' indicate that

rather more than half of the 'extra N' was derived from the protein and nucleic acid lost from the liver. If the surprisingly high value of Exp. 3 is included, the mean of the ratios is 0.68, and without it, 0.59. Non-protein N and lipid N, amounting to approximately 10% of the protein + nucleic acid N, will further increase the ratios. Thus, even if the high coefficient of variation is taken into consideration, at least 60% of the 'extra N' excreted in the urine during the first 5 days of a protein-free diet was accounted for by the N lost from the liver during this period.

Table 2. *Protein + nucleic acid N in livers of rats fed on different diets*

Diet	No. and sex of rats	Protein + nucleic acid N (mean and s.e. of the mean) (mg./100 g. initial body weight)
Stock	7 ♀	105.9 $\pm$ 2.0
4 days protein-free (starch)	4 ♀	78.0 $\pm$ 1.7
7 days protein-free (starch)	4 ♀	76.2 $\pm$ 1.7
5 days protein-free (sucrose)	5 ♀	78.0 $\pm$ 1.5
Stock	4 ♂	110.0 $\pm$ 2.1
7 days 54% casein	4 ♂	129.6 $\pm$ 5.8
5 days protein-free	4 ♂	70.5 $\pm$ 1.6

Daily variations of 'extra N' in urine and of losses of protein and nucleic acid N from the liver (Figs. 1-3). The curves obtained for the daily excretions of total urinary N in Exps. 1, 3 and 4 were not inconsistent

with the results in human beings of Martin & Robison (1922), who found that the excretions of 'extra N' in the urine diminished exponentially during the first few days of a protein-free diet. On the other hand, the curve of Exp. 2 is rather irregular.

Fig. 1. Excretion of total urinary N in female rats fed on a protein-free diet. (The curves were fitted by eye.) The vertical lines represent twice the standard error of the mean and in some instances, in order to avoid confusion, were drawn in one direction only.

The daily losses of protein and nucleic acid N from the liver were calculated from the regression equations for labile N (mg./100 g. body weight) on days of protein-free diet: $N_t = 31.7 (0.33)^t$ for female rats, and $N_t = 42.5 (0.60)^t$ for male rats. These equations were obtained from the regression equations for labile non-glycogen, non-lipid liver

The values obtained in Exps. 1 and 3 for 'extra N'/24 hr. in urine and for protein + nucleic acid N lost from the livers in 24 hr. are plotted in Fig. 3.

Fig. 2. Excretion of total urinary N in male rats fed on a protein-free diet. (The curves were fitted by eye.) The vertical lines represent twice the standard error of the mean.

In Exp. 1 (female rats) the two curves run close together with the exception of the value for the first day, while they show a considerable discrepancy in

Fig. 3. Excretion of 'extra N' and loss of N from the liver. (The curves for 'extra N' were fitted by eye and those for loss of liver N were calculated from regression equations.)

solids (mg./100 g. body weight) on days of protein-free diet: $NNS_t = 254 (0.33)^t$ for female rats, and $NNS_t = 352 (0.60)^t$ for male rats. The constants 254 and 352 were multiplied by 0.916×0.136 and 0.885×0.136 respectively; the factors 0.916 and 0.885 convert NNS to protein + phospholipin + nucleic acid, and the factor 0.136 converts the latter to protein + nucleic acid N (Campbell & Kosterlitz, 1948a, c).

Exp. 3 (male rats). Whether this difference between male and female rats is significant cannot be decided with the relatively few data at present available.

DISCUSSION

The lag in the fall of urinary N to a new equilibrium when dogs were fasted, or when their protein intake was reduced, induced Voit (1866, 1881) to formulate

his theory of 'circulating protein'. This was supposed to serve as a reserve pool for protein, which was utilized by the tissues. These fundamental observations have been amply confirmed by numerous workers and extended to animals of many species, either fasted or fed on protein-free diets. Rubner (1911) thought that the 'extra N' excreted during the first few days of a protein-free diet was due to 'storage protein', which was retained or lost within 3-5 days after increasing or decreasing the protein intake. Storage protein was assumed to be unorganized, and to have as its main function the maintenance of nitrogen equilibrium with an intermittent food intake.

The experiments described in this paper, however, indicate that, while a given quantity of 'extra N' appears in the urine, at least 60%, and probably more, of this amount of N is lost from the liver. It is of interest to note that both the losses of nitrogen from the liver (Campbell & Kosterlitz, 1946, 1948*a*) and the excretions of 'extra N' (Martin & Robison, 1922) diminish exponentially during the first few days of a protein-free diet. The fact that no other organ loses nitrogen at a rate comparable with that of liver during 2 days of fasting or of a protein-free regimen (Addis, Poo & Lew, 1936*a, b*) supports the view that most of the 'extra N' is derived from the liver.

Conversely, Addis *et al.* (1936*b*) found that, in rats which had been transferred from a protein-free to a 74% casein diet, the first definite increase in liver protein occurred as early as 17 hr. after the

protein feed. Campbell & Kosterlitz (1948*a*), who depleted rats of their labile liver cytoplasm by a protein-free regimen lasting 4 days and then fed them on an 18% casein diet, found that more than half of their labile liver cytoplasm was restored in 24 hr. and that the recovery was virtually complete in 48 hr. These findings suggest that the retention of urinary nitrogen during a period of increased protein intake can at least partly be ascribed to the formation of labile liver cytoplasm.

SUMMARY

1. An attempt has been made to correlate the excretion of 'extra N' in the urine of rats during the first few days of a protein-free regimen with the nitrogen lost from the livers during the same period.

2. At least 60% of the 'extra N' can be accounted for by the nitrogen derived from the protein, nucleic acid and other nitrogenous substances of the liver.

3. These findings make unlikely the older assumption of an unorganized 'circulating' or 'storage' protein which was supposed to be responsible for the appearance of the 'extra N'.

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