Biochemical Pharmacology, Vol. 16, pp. 590–592. Pergamon Press Ltd. 1967. Printed in Great Britain

Urinary excretion of inorganic phosphates in rats after a single injection of chloroquine sulphate

(*Received* 24 *October* 1966; *accepted* 3 *November* 1966)

ANTIMALARIAL drugs are used for treatment of many diseases. Chloroquine was used for instance, for treatment of collagenous diseases, some allergic diseases, some kidney diseases and cardiac arrhytmia. Chloroquine was also used for treatment of hepatitis epidemica,[18] lupus erythrematosis[11] and bronchial asthma.[8] Chlororquine produces undesirable reactions: headache, dizziness, dyspepsia, ocular disturbances, psychoses etc.[2, 17] Cardiovascular effects of chloroquine with special reference to its antifibrillatory action were described.[12] Also chloroquine psychoses[19] and chloroquine psychotrophic effects were described.[21]

Chloroquine is slowly metabolized in the human body, essentially by oxidation of the side chain with 4-amino-7-chlorochinoline as a result[15]. Chloroquine's metabolite 4-amino-7-chlorochinoline influenced *in vivo* oxidative phosphorylation[20]. From this point of view it could be possible that changes may occur in the metabolism of phosphates in the body.

Thus experiments were carried out to determine the urinary erection of inorganic phosphates after a single injection of chloroquine.

MATERIALS AND METHODS

Urinary excretion of inorganic phosphates were determined in rats after a single intraperitoneal injection of chloroquine sulphate* in a dose of 20 mg/kg.

Male Wistar albino rats were used. Experiments were carried out in groups of rats weighing approximately 150 g (A), 120 g (B), 240 g (C) and 300 g (D).

(A) There were experimental and control groups with eight rats weighing 150 g in each. Experimental rats were intraperitoneally injected with sterilized chloroquine sulphate solution in a dose of 20 mg/kg of body weight. Controls received the same volume of physiological saline. The rats were kept in metabolic cages and water was given *ad libitum*. Urine samples were collected in calibrated test tubes at 6, 12 and 24 hr. The volume of water drunk and the volume of urine excreted were measured at each interval.

After centrifugation of the urine samples, inorganic phosphates were determined using ammonium molybdate reagent and L-ascorbic acid as reducing agent. The extinction was measured at 700 mμ in a Beckman spectrophotometer. The method of Chen, Toribara, and Huber[13] was used.

The urine was dried in small weighed aluminium dishes in an electric oven at 105° to constant weight. Dry matter was determined. The amount of inorganic phosphate was calculated per 1 g of dry matter.

(B) There were experimental and control groups with six rats each weighing 120 g.

(C) There were experimental and control groups with six rats each weighing 240 g.

(D) There were experimental and control groups with six rats each weighing 300 g.

RESULTS

(A) Single injection of chloroquine sulphate in a dose of 20 mg/kg of body weight produced in rats weighing 150 g, significant increase in inorganic phosphates in the urine in 6 hr, Fig. 1. There was a slight decrease of inorganic phosphates at the 12 hr interval which was not significant. At the 24 hr interval there was a slight increase in organic phosphates which was also not significant.

Water consumed increased slightly at all intervals but none of these differences were significant. The volume of urine excreted increased slightly at each interval, but there were no significant differences.

(B) In rats weighing 120 g there were the same results as in experiment (A).

(C), (D) In rats weighing 240 g and 300 g there were no significant differences in organic phosphates in the urine.

* Chloroquine sulphate injection B.P. ("Nivaquine", May & Baker), containing the equivalent of 40 mg base ml solution was used.

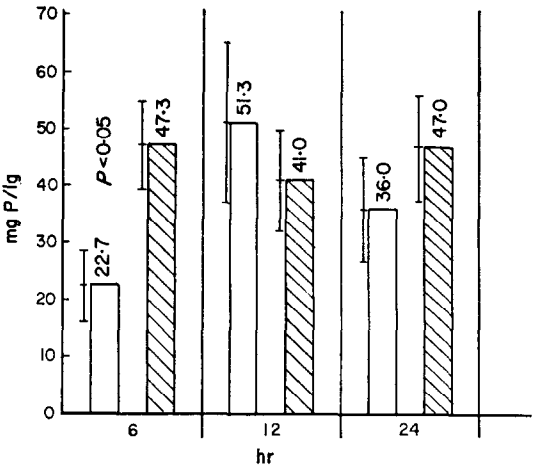

FIG. 1. Urinary excretion of inorganic phosphates in rats after a single injection of chloroquine sulphate (20 mg/kg of body weight). On the ordinate: mg of phosphates per 1 g of dry matter in the urine. On the abscissa: time in hours. Open column: controls; shaded column: injected rats.

In rats weighing 240 g there were no changes in volume of water drunk and urine excreted in 6 hr Other intervals at 12 and 24 hr were not carried out.

In rats weighing 300 g there were no changes in water intake at 6 hr (controls: 1, 8 ml; experimental: 1, 5 ml) and 12 hr (controls: 7, 2 ml; experimental: 6, 0 ml). There was significant increase at the 24 hr interval (controls: 5, 6 ml; experimental: 15 2 ml. $P < 0, 05$).

There were no differences in the volume of urine excreted at the 6 hr (controls: 3, 5 ml; experimental: 2, 8 ml) and the 12 hr (controls: 5, 5 ml; experimental: 5, 1 ml) intervals. There was significant increase at the 24 hr interval (controls: 4, 7 ml; experimental: 9, 3 ml. $P < 0, 05$).

DISCUSSION

Increased urinary excretion of inorganic phosphates in young rats after a single injection of chloroquine sulphate gives evidence that changes in the phosphate metabolism in the body occur, Urinary excretion of inorganic phosphates was followed because the organism predominantly excretes phosphorus in the form of sodium salts (95 per cent) in the urine[7]. Phosphates utilization in the body is influenced by many factors. Protein deficiency in the diet stimulates phosphorus metabolism in the rat liver.[9, 10] There are differences in the urinary excretion by infants and by adults and the daily rhythm also influences the phosphate levels in the urine.[4] All experiments were done each day at the same time.

The content of phosphorus compounds in the body depends on age. For instance, the content of inorganic phosphate in skeletal muscle was 56,8 for young rats, 35,2 for mature rats, and 32,9 mg per cent for old rats.[5] Recently evidence was given about influence of vasopressin on the urinary excretion of phosphorus.[14]

Antirheumatic drugs produce inhibition of phosphorylation processes in the body due to uncoupling effect.[3] Adams and Colb[1] showed that there exists a parallel between anti-inflammatory effect, increased oxygen intake and uncoupling of oxidation from phosphorylation. It has been observed that antimalarial drugs antagonize some pharmacological actions of adensoine, a constituent of some coenzymes, and that the antiplasmodial activity runs parallel with this antagonistic effect. Chloroquanidin antagonizes adenosine only after it has been converted into an active metabolite in the blood.[6]

The inhibitory factor present in the blood of malaria-infected animals has been shown to inhibit the *in vitro* respiration and oxidative phosphorylation of isolated normal liver cell mitochondria.[16]

SUMMARY

The compound 4-amino-7-chlorochinoline, a product of chloroquine oxidation,[15] also influence oxidative phosphorylation.[20]

Chloroquine or its metabolites play some role in phosphorus metabolism in the body.

Urinary excretion of inorganic phosphates in rats weighing 150 g, 120 g, 240 g and 300 g after a single intraperitoneal injection of chloroquine sulphate in a dose of 20 mg/kg of body weight was determined at the intervals of 6, 12, and 24 hr. Volumes of water drunk and urine excreted were measured.

In rats weighing 150 g and 120 g there was a significant increase in inorganic phosphates in the urine at the 6 hr interval after an injection of chloroquine sulphate. There were no significant differences in the volumes of water drunk and urine excreted.

In rats weighing 240 g and 300 g there were no significant differences in inorganic phosphates in the urine at all intervals.

In rats weighing 300 g there was a significant increase in water intake and in the volume of urine excreted at the 24 hr interval.

Increased urinary excretion of inorganic phosphates after an injection of chloroquine sulphate in young rats gives evidence that changes in phosphate metabolism occur, while in old rats changes in water metabolism occur.

Chloroquine or its metabolites can influence phosphorus metabolism of the body.

National Institute of Health and Medical Research, Accra, Ghana

JAROSLAV KOLOUŠEK*

* Address: P.O. Box 2848, Accra-Ghana.

REFERENCES

1. S. S. ADAMS and R. COLB, *Nature, Lond.* **181**, 773 (1958).
2. F. E. BERGLOF, *Acta Rheum. Scand.* **7**, 83 (1961).
3. T. M. BRODY, *J. Pharmacol.* **117**, 39 (1956).
4. R. F. A. DEAN, *J. Physiol.* **107**, 182 (1948).
5. E. V. EPSTEIN, *Geriatrics Mechanisms*, in Russian, p. 101, Gos. Izdat. Med. Lit., Kiev, USSR (1963).
6. L. S. GOODMAN and A. GILMAN, *The Pharmacological Basis of Therapeutics*, p. 1167, 2nd ed., Macmillan, New York (1958).
7. A. A. GORODECKII, T. P. SIVACHENKO, O. A. KHOMUTOVSKII and E. Z. RIABOVA, *Videlenie iz organizma nekotorikh radio aktivnikh vescestv*, in Russian, p. 21, Gos, Med. Izdat., Kiev, USSR (1959).
8. P. GREGORI, *Dte GesundhWes.* **18**, 1373 (1963).
9. G. E. GRODZENSKII and E. I. KOROLEVA, *Biokhimia* **14**, 35 (1949).
10. G. E. GRODZENSKII and E. I. KOROLEVA, *Biokhimia* **14**, 511 (1949).
11. CH. GRUPPER, *Thérapie* **18**, 1177 (1963).
12. M. E. HESS and C. F. SCHMIDT, *Circulation Res.* **7**, 86 (1959).
13. P. S. CHEN, T. Y. TORIBARA and W. HUBER, *Analyt. Chem.* **28**, 1756 (1956).
14. J. KOLOUŠEK, *Archs int. Physiol. Biochim.* **74**, 276 (1966).
15. K. KURODA, *J. Pharmacol.* **137**, 156 (1962)l
16. B. G. MAEGRAITH and K. A. FLETCHER, *Progress in Protozoology*. Abstracts of Papers read at the Second Intern. Conference of Protozoology, London, 29th July–5th August (1965). International Congress Series No. 91, p. 167. Excerpta Med. Foundation, Amsterdam (1965).
17. I. I. MAKARENKO and E. R. LEVICKII, *Sov. Med.* **27**, 72 (1964).
18. W. RUMLER, *Dte GesundhWes.* **20**, 1003 (1965).
19. O. L. SAPP, *J. Am. Med. Ass.* **187**, 373 (1964).
20. M. W. WHITEHOUSE and H. BOSTRÖM, *Biochem. Pharmac.* **14**, 1173 (1965).
21. M. YVONNEAU and N. FELDMAN, *Presse méd.* **73**, 705 (1965).