Biochemical Pharmacology, Vol. 29, pp. 1729–1735.

0006-2952/80/0615-1729 $02.00/0

CHLOROQUINE INHIBITS THE INSULIN PRODUCTION OF ISOLATED PANCREATIC ISLETS

ARNE ANDERSSON,* STEN OLSSON† and HANS TJÄLVE†
*Departments of Histology and †Toxicology, University of Uppsala, Uppsala, Sweden

(*Received* 27 *December* 1979; *accepted* 19 *February* 1980)

Abstract—It has recently been demonstrated that there is a high and persistent uptake of chloroquine in the islets of Langerhans as shown by both *in vivo* autoradiographical and *in vitro* uptake studies. The present study aimed at an evaluation of the possible functional effects, which might result from the accumulation of chloroquine in the islet cells. It was found, in short-term experiments, that 10^{-4}M chloroquine abolished the glucose-stimulated (pro)insulin biosynthesis of isolated mouse islets and also impaired the production of $^{14}CO_2$ from (^{14}C)glucose. However, a slight enhancement of glucose-stimulated insulin release was observed both *in vitro* and *in vivo*. Furthermore, long-term experiments performed by adding 10^{-5}M chloroquine to the RPMI 1640 medium used for culture of isolated islets resulted in a substantial but reversible inhibition of the (pro)insulin biosynthesis. Culture in the presence of chloroquine also reduced both the oxidative rate of glucose and the insulin content of the islets, and abolished glucose stimulation of the insulin release. Electron microscopy showed that both multilamellated bodies and autophagic vacuoles were induced in the B cells cultured in the presence of chloroquine. The present data suggests that chloroquine, at doses similar to those used in long-term treatment, markedly impairs the specific functions of the insulin producing B cells.

Besides its use as an antimalarial agent, chloroquine is widely used in long-term treatment of collagen diseases. It has been demonstrated autoradiographically that there is a strong accumulation and prolonged binding of the drug in some endocrine cell systems including the pancreatic islets [1]. Indeed, recent *in vitro* studies indicated that chloroquine is extensively taken up by the islet cells [2]. It was of interest, therefore, to examine to what extent this accumulation process was accompanied by effects on the structure and function of the islets. In the present study, collagenase-isolated islets were utilized to investigate the acute effects of the drug on the insulin production of the islet B cells. Furthermore, islets were maintained in tissue culture for one week in the presence of the drug, allowing a detailed characterization of the long-term effects of chloroquine on different aspects of islet cell structure and function.

MATERIALS AND METHODS

Chemicals. Collagenase was purchased from Worthington Biochemical Corp., Freehold, NJ, and chloroquine diphosphate from Sigma Chemical Co., St. Louis, MO. L-(2,4,6-^{3}H)-phenylalanine (sp. radioactivity 62 Ci/mmole) and ^{125}I-insulin were obtained from The Radiochemical Centre, Amersham, U.K. and ^{125}I-glucagon from Novo Industri A/S, Copenhagen, Denmark.

Animals. Male NMRI-mice (Anticimex, Sollentuna, Sweden) weighing 25–30 g were used throughout the study. All animals were fed a standard pellet diet (AB Ewos, Södertälje, Sweden), but were starved overnight prior to the *in vitro* experiments.

In vitro *experiments.* Pancreatic islets were isolated by collagenase digestion and cultured as described previously [3]. The islets were cultured in a free-floating manner and the standard culture medium was RPMI 1640 containing 11 mM glucose, to which was added 10% (v/v) calf serum, antibiotics and chloroquine diphosphate as given below. The medium was changed on the third and then every second day of culture. The methods used for the measurements of the hormone content, the insulin release in batch type incubations and the insulin biosynthesis in the islets have been reported previously [3]. [^{3}H]-phenylalanine was, however, used as the radioactively labelled precursor in the present studies. Islet perifusion experiments to determine the dynamics of insulin secretion were performed as described previously [4]. Concentrations of insulin and glucagon in both extracts and incubation media were measured by radioimmunoassay procedures [5, 6]. Glucose oxidation rates were estimated essentially as described by Andersson [7].

Electron microscopy. Pancreatic islets were cultured, as described above, in the presence or absence of 10^{-5}M chloroquine. The islets were subsequently fixed in 3.5% glutaraldehyde and postfixed in 2% osmium tetroxide. After dehydration in alcohol the islets were embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate and examined in a JEOL-JEM 100B-electron microscope.

In vivo *experiments.* The orbital vein plexus of non-fasted animals was punctured in order to obtain a blood sample prior to the injection of glucose into a tail vein. The glucose was injected as a 30% solution to give a dose of 3.75 g/kg body wt, to which chloroquine was added, at a concentration of 15 or 30 mg/kg body wt. Another blood sample was collected 5 min after the injection. After coagulation and centrifu-

gation the serum was removed and stored at −20° until assayed for glucose and insulin. Glucose was measured using a Beckman Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, CA).

Expression of results. The results are given as means ± S.E.M. with the number of experiments given within parentheses. For statistical analysis, Student's *t*-test for paired or non-paired data was used.

RESULTS

Effects in vitro *on non-cultured islets*

Insulin release. The effects of the drug on glucose-stimulated insulin secretion were examined in a perifusion system. Addition of 10^{-4}M chloroquine to high concentrations of glucose was found to slightly enhance the second phase of insulin secretion ($P < 0.05$), whereas no effect was recorded on the first peak (Fig. 1). Withdrawal of the drug in combination with a return to low glucose concentrations was followed by a prompt return to the basal secretion rate.

Insulin biosynthesis. As shown in Table 1, 10^{-4}M chloroquine decreased the incorporation of [^{3}H]-phenylalanine, at 10 mM glucose, into both (pro)insulin and TCA-precipitable islet proteins. The former process was, however, inhibited to a greater degree, since the figure expressing insulin biosynthesis as a percentage of total protein synthesis was also markedly depressed. This was also the case for islets incubated in the presence of 10^{-5}M chloroquine, whereas no effects were recorded with the lowest concentration of the drug tested.

Glucose oxidation. Chloroquine (10^{-4}M) significantly impaired the production of $^{14}CO_2$ from [^{14}C]-glucose. Thus, a glucose oxidation rate, at 16.7 mM glucose, of 407 ± 39 was reduced to 302 ± 57 pmoles/90 min per 10 islets when the drug was added to the oxidation medium ($P < 0.05$; $N = 10$).

Effects on islets in culture

Ultrastructural studies. Control islets cultured for one week were structurally well preserved, although the B cells were slightly degranulated. Addition of 10^{-5}M chloroquine to the culture medium produced dramatic ultrastructural changes in the islet B cells, the most conspicuous of which was the presence of so called 'myeloid bodies' (Fig. 2). There were also many autophagic vacuoles containing electron dense bodies, some of which could be recognized as B cell granules. In addition, intact myeloid bodies were occasionally found in these vacuoles. We have, so far, been unable to locate myeloid bodies in the other types of islet cells. The presence of the myeloid bodies seemed to be a reversible phenomenon, since prolongation of the treatment culture period to include a recovery period of four days without the drug was followed by an almost complete disappearance of these structures. The mitochondria of the chloroquine-treated islet B cells were of normal appearance and the cytoplasm contained rough endoplasmic reticulum in vacuolar form. Moreover, the B cells were markedly degranulated and sometimes even completely devoid of granules.

Hormone content and release. As seen in Fig. 3, the insulin content of islets exposed to 10^{-5}M chloroquine for one week *in vitro* decreased to about 5 per cent of that in the control islets ($P < 0.001$). No effect of exposure to the drug was seen on the islet glucagon content. The effects of glucose on insulin secretion were recorded in short-term incubations,

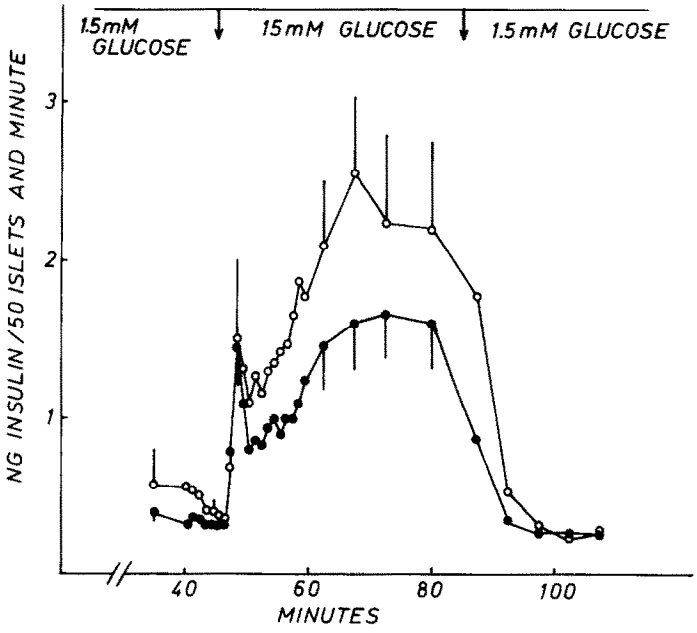

Fig. 1. Effects of 15 mM glucose (●) or 15 mM glucose + 10^{-4}M chloroquine (○) on the insulin secretion by 50 freshly isolated mouse pancreatic islets. The results are given as the mean ± S.E.M. of seven experiments. In each experiment the mean of the insulin concentrations of the last four fractions during the glucose stimulation was calculated. The statistical significance of the differance obtained by the addition of chloroquine was tested by means of a paired *t*-test.

Table 1. Effects of chloroquine on glucose-stimulated insulin biosynthesis in freshly isolated islets*

Additions	PI-I	TCA	%	
None	15.3 ± 1.9	50.4 ± 5.0	30.4 ± 1.6	(7)
Chloroquine (10^{-4}M)	2.5 ± 0.6‡	19.8 ± 2.8†	13.5 ± 1.3‡	(7)
Chloroquine (10^{-5}M)	11.4 ± 1.7	47.2 ± 6.1	23.9 ± 1.3†	(7)
Chloroquine (10^{-6}M)	15.2 ± 2.4	57.5 ± 8.4	26.1 ± 1.9	(5)

* Insulin biosynthesis was determined by measuring the incorporation of [^{3}H]-phenylalanine, at 10 mM glucose, into both the (pro)insulin (PI-I) and TCA-precipitable protein fractions (TCA) of islet homogenates. Results are expressed as c.p.m. × 10^{-3}/2 hr/10 islets. 45 c.p.m. × 10^{-3} corresponds to 1 pmole of phenylalanine. The values in column (%) refer to the percentage of the total incorporated radioactivity represented by the PI-I fraction as calculated from each individual observation. Means ± S.E.M. are given, together with the statistical significance of the difference obtained by the addition of chloroquine. Number of experiments are given within parentheses.

† $P < 0.01$.

‡ $P < 0.001$.

at the end of the culture period (Fig. 3), and it was found that control islets responded with a 10-fold increase in insulin release when challenged with high concentrations of glucose ($P < 0.005$). The drug exposed islets, however, were totally unresponsive to the stimulation of high glucose concentrations.

Insulin biosynthesis. The incorporation of [^{3}H]-phenylalanine into (pro)insulin or TCA-precipitable proteins of one week cultured islets was estimated at 10 mM glucose and in the absence of the drug (Table 2). Addition of chloroquine to the culture medium did not affect total protein biosynthesis, whereas a clear-cut decrease in (pro)insulin biosynthesis was observed after culture in the presence of 10^{-5}M chloroquine. Culture with a 10-fold lower concentration of the drug resulted in a small, although not statistically significant, decrease in (pro)insulin biosynthesis. The percentage figure giving the ratio between the radio activity incorporated into (pro)insulin and total proteins was, however, decreased, suggesting that the protein biosynthesis in these islets was directed, to a lesser extent, towards (pro)insulin biosynthesis even after culture at this low chloroquine concentration.

The reversibility of this impairment of (pro)insulin biosynthesis was examined in a separate series of experiments (bottom part of Table 2). Findings indicated that withdrawal of chloroquine from the culture medium after one week's culture resulted in a substantial recovery of the biosynthetic rate.

Glucose oxidation. The production of $^{14}CO_2$ from [^{14}C]-glucose at 16.7 mM glucose was significantly reduced after one week's culture in the presence of 10^{-5}M chloroquine. The oxidative rates were 271 ± 45 for the drug exposed islets and 664 ± 69 pmoles/90 min per 10 islets for the controls ($P < 0.001$; $N = 6$).

Effects on insulin secretion in vivo

The *in vivo* studies of the acute effects of chloroquine on insulin secretion showed that the peak levels of serum insulin observed 5 min after the injection of glucose were higher, when chloroquine, at a dose of 30 mg/kg body wt, had been added to

Table 2. Insulin biosynthesis in cultured islets*

Culture conditions	PI-I	TCA	%	
RPMI 1640	23.3 ± 3.1	113.6 ± 13.0	20.2 ± 1.7	(6)
RPMI 1640 + 10^{-5}M chloroquine	5.0 ± 0.7†	128.8 ± 21.5	4.3 ± 0.8†	(6)
RPMI 1640 + 10^{-6}M chloroquine	18.8 ± 2.6	124.4 ± 15.5	14.7 ± 1.2‡	(6)
RPMI 1640 cultured for 11 days	20.9 ± 3.3	101.5 ± 11.7	20.7 ± 2.2	(6)
RPMI 1640 + 10^{-5}M chloroquine for 7 days and without chloroquine for another 4 days	13.0 ± 3.5	70.9 ± 12.7	17.6 ± 2.3	(6)

* Islets were cultured for 7 (upper panel) or 11 days (bottom panel) in the absence or presence of chloroquine. Insulin biosynthesis was estimated at 16.7 mM glucose and in the absence of the drug. For further details see legend to Table 1.

† $P < 0.001$.

‡ $P < 0.05$.

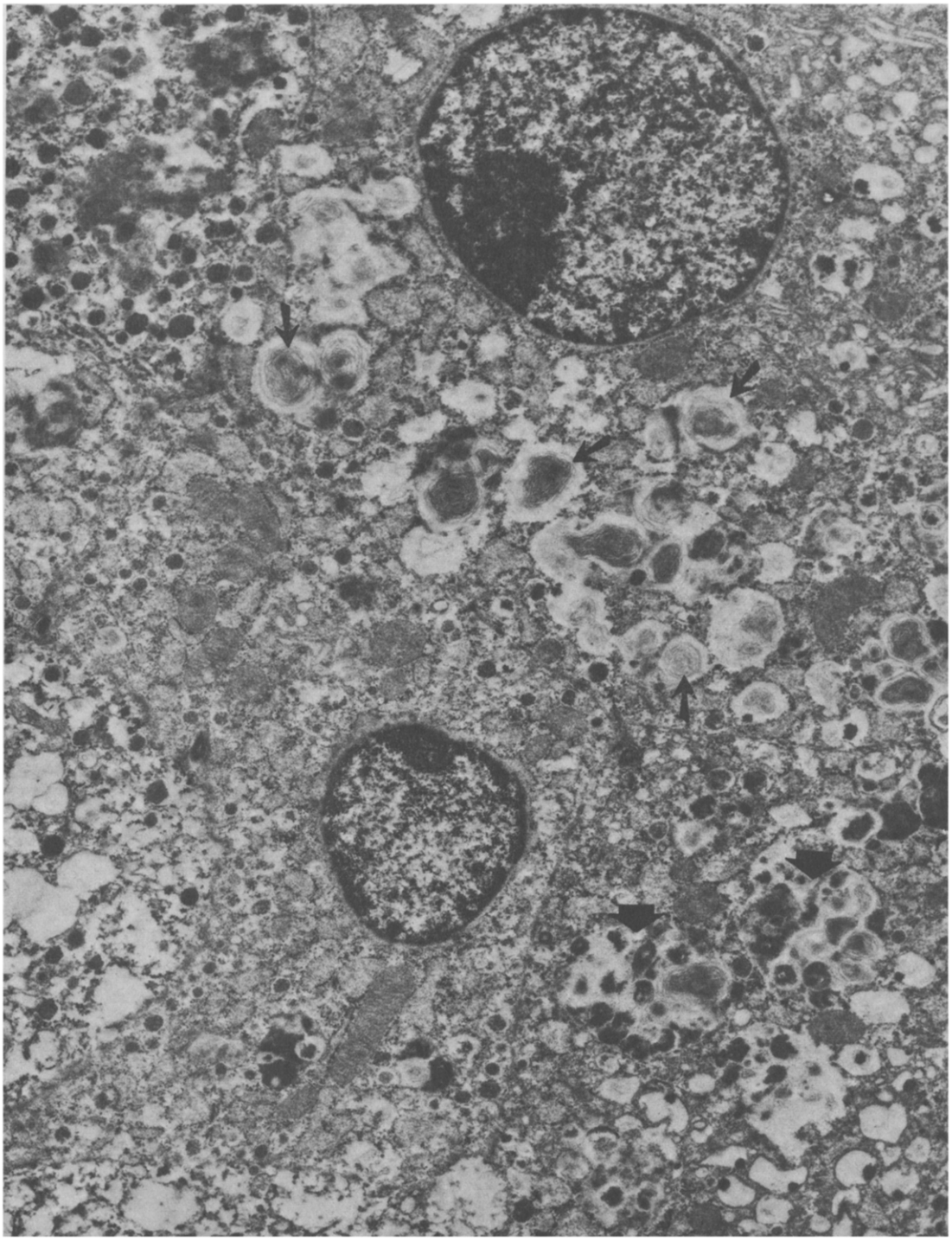

Fig. 2. Electron micrograph of an islet cultured for seven days in the presence of 10^{-5}M chloroquine. Several myeloid bodies (thin arrows) are present in the B cell in the upper right of the picture. The B cell in the lower right corner shows autophagic vacuoles (thick arrows) containing myeloid bodies and B cell granules with disintegrated cores. In the centre is a B cell with more normal morphology, although it contains a few autophagic vacuoles. In the upper left corner is a part of well granulated A_2-cell (×8.1).

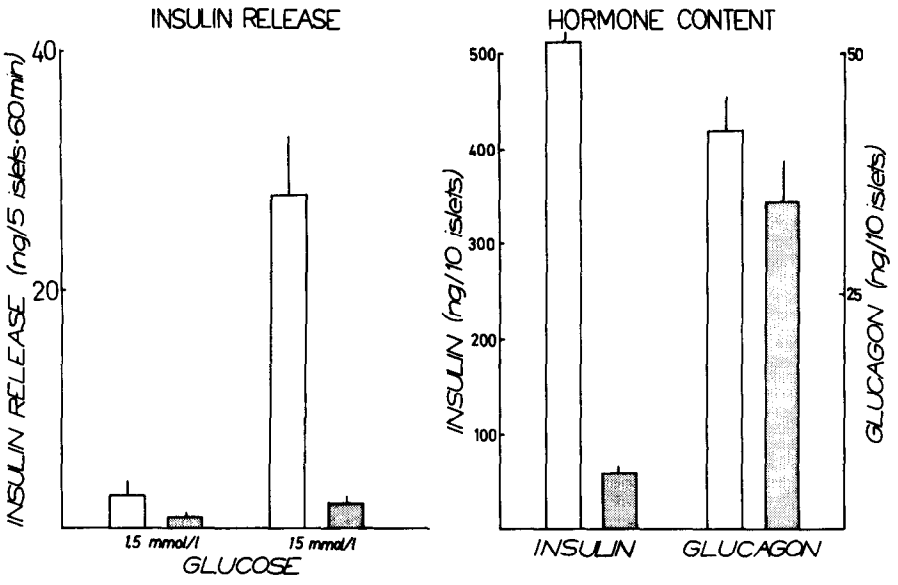

Fig. 3. Islets were cultured for one week in the absence (open bars) or presence of 10^{-5}M chloroquine (stippled bars). The insulin release (means ± S.E.M. of five experiments) was estimated in short-term incubations performed either in 1.5 or 15 mM glucose. Insulin and glucagon content was estimated in acid ethanol extracts of the islets at the end of the culture period (means ± S.E.M. of twelve experiments).

Table 3. Effects on serum glucose and insulin of two doses of chloroquine injected i.v. together with glucose*

Agents	Se-glucose 5′	Se-insulin 0′	Se-insulin 5′	Per cent increase
Glucose (3.75 g/kg)	36.1 ± 2.1	2.16 ± 1.09	5.25 ± 1.21†	407 ± 108
Glucose (3.75 g/kg) + chloroquine (15 mg/kg)	34.8 ± 3.1	1.74 ± 0.62	7.10 ± 1.46‡	504 ± 101
Glucose (3.75 g/kg) + chloroquine (30 mg/kg)	39.6 ± 0.8	1.46 ± 0.33	9.70 ± 0.82†	905 ± 230§

* Serum glucose (mmoles/1) and serum insulin (ng/ml) values are expressed as means ± S.E.M. of 5 experiments, each of which is based on observations on three mice. Blood samples were taken before (0′) or 5 min (5′) after the injection. The statistical significance of the difference in serum insulin before and after the injection is given (†‡) as well as that obtained by the addition of chloroquine to the glucose solution. (§)

† $P < 0.01$.

‡ $P < 0.05$.

§ $P < 0.05$.

the glucose solution (Table 3). No statistically significant effects were recorded in experiments performed using a dose of 15 mg/kg body wt.

DISCUSSION

This study was performed to determine whether the *in vivo* [1] and *in vitro* [2] uptake of chloroquine by the islets of Langerhans was accompanied by structural or functional changes. Indeed, the present results indicate that long-term *in vitro* exposure of pancreatic islets to chloroquine mimics the typical drug-induced ultrastructural appearance with myeloid bodies [8] and autophagic vacuoles [9]. Our findings are also in agreement with the recent report of similar morphological changes in monolayer cultures of rat endocrine pancreas cells exposed to chloroquine [10]. In the present investigation, however, we were unable to demonstrate myeloid bodies in the glucagon containing A_2-cells. In support of this finding, the glucagon content of the islets cultured in the presence of chloroquine remained the same as the controls. Extended studies, using for instance A_2-cell-rich islets prepared from streptozotocin-treated guinea pigs [11, 12], are, however, needed before it can be resolved whether or not the A_2-cells are affected by chloroquine treatment *in vitro*. It is of interest that ultrastructural changes, similar to those observed in this study, have been reported in experiments with cyproheptadine exposure of rat islets maintained in tissue culture [13–15].

In addition to these ultrastructural changes, marked functional effects of chloroquine were observed in both the short- and long-term experiments. The short-term experiments showed an inhibition of the protein biosynthesis of the pan-

creatic islets. Inhibitory effects on protein biosynthesis by chloroquine have been reported earlier in other systems. Thus, Roskoski and Jaskunsas [16] showed that chloroquine inhibits polypeptide synthesis in rat liver cell-free systems, probably by interacting with the free polynucleotide and preventing the subsequent formation of an active polynucleotide–ribosome complex. Moreover, Lefler *et al.* [17] demonstrated an inhibition of aminoacylation and polypeptide synthesis by chloroquine in subcellular rat liver systems. Chloroquine has also been shown to inhibit protein biosynthesis in bovine [18] and rabbit [19] retinal preparations. Our present data indicates that, in the pancreatic islets, proinsulin biosynthesis is particularly sensitive to the chloroquine effect, since incorporation of [^{3}H]-phenylalanine into proinsulin was reduced to less than 20 per cent of that of the controls, the corresponding figure for total protein biosynthesis being 40 per cent. A few mechanisms by which chloroquine has been proposed to inhibit protein biosynthesis have been mentioned above. However, there is evidence that chloroquine may act on several cell sites by virtue of its marked ability to bind to tissue macromolecules, such as nucleic acids, proteins and phospholipids [20–22]. The inhibition of the protein biosynthesis in the islets may therefore be the result of a multifactorial process.

Our experiments showed a marked decrease in the rate of glucose oxidation in the islets. It is possible that this may be due to an inhibition of mitochondrial electron transport, an effect which chloroquine has been shown to exert both on ox heart mitochondria [23] and mitochondria prepared from muscle tissue of *Ascaris suum* [24]. The reduction in energy sources resulting from the decreased rate of glucose oxidation in freshly isolated as well as cultured islets may be another mode of action by which chloroquine may have exerted its inhibitory effects on glucose-stimulated insulin biosynthesis. Against this background, it is, however, difficult to explain our present *in vitro* and *in vivo* results, suggesting that chloroquine enhances glucose-induced insulin release, since the latter process is thought to be mediated by a sequence of metabolic, ionic and motile events (for review, see ref. 25). One possible explanation for the drug-induced potentiation of insulin secretion may be a direct membrane effect. Our recent uptake studies [2] indicated a non-energy dependent binding of chloroquine to cellular constituents, such as the phospholipid components of the membranes, as a possible mechanism for the accumulation of the drug in the islets. Studies by DiDonato *et al.* [26] indicated that as much as one-fourth of the total uptake of chloroquine by human fibroblasts was bound to the cell surface. It has been known for a long time that many agents, for instance sulfhydryl reagents such as chloromercuribenzene-*p*-sulfonic acid, stimulate insulin secretion by a direct membrane action [27].

The data from our culture experiments clearly indicated that the insulin content, release and biosynthesis of the chloroquine-exposed islets were all markedly depressed despite the fact that the drug was absent during the short-term experiment performed at the end of the culture period. The total protein biosynthesis, however, appeared to be unaffected in the exposed islets, suggesting a drug-induced 'switch' in the activities of the B cells from insulin biosynthesis to production of other proteins. The observed ultrastructural alterations support this proposal. There is clear morphological and biochemical evidence that the drug is accumulated in the vacuoles of the cytoplasm and that these are of a lysosomal nature [9, 28]. Chloroquine may be trapped by protonation in the acid milieu of the lysosomes by virtue of its weak basic properties [20]. There is a subsequent increase in the intralysosomal pH by consumption of hydrogen ions and this in turn may induce alterations of lysosomal function [28–30] with autophagy as the ultimate result. It seems, however, unlikely that some kind of a lysosomal dysfunction could be responsible for the chloroquine-induced impairment of insulin production in the short-term experiments. On the other hand, such mechanisms could well contribute to the inhibition of insulin biosynthesis in the islets exposed to chloroquine during the one week culture period. The present observation of a recovery of both the insulin production and the morphological appearance of the islets within a few days of removal of the drug, however, suggests that the drug-induced effects are reversible.

Extended *in vivo* studies will be required before the possible clinical implications of the present data can be evaluated. It is worthy of note, however, that the serum chloroquine concentration of patients suffering from side effects is only slightly above 10^{-6}M [31]. Addition of a similar concentration of the drug to culture medium RPMI 1640 effected a clear-cut impairment of islet B cell function (cf. Table 2). There are to our knowledge, however, no reports suggesting a decreased glucose tolerance or an increased frequency of diabetes mellitus in patients being treated for long periods of time with high doses of chloroquine.

Acknowledgements—The expert technical assistance of Mrs. Margareta Engqvist, Miss Ewa Hansson, Miss Astrid Nordin and Mrs. Parri Wentzel is gratefully acknowledged. This work was supported by financial grants from the Swedish Medical Research Council (12X-109), The Nordic Insulin Fund and the Swedish Diabetes Association.

REFERENCES

1. L. Dencker, N. G. Lindquist and H. Tjälve, *Med. Biol.* **54**, 62 (1976).
2. H. Tjälve, S. Olsson and A. Andersson, *Acta pharmac. tox.*, in press.
3. A. Andersson. *Diabetologia* **14**, 397 (1978).
4. A. Andersson, K. Asplund and R. Larkins, *Acta physiol. scand.* **104**, 377 (1978).
5. L. Heding, *Diabetologia* **8**, 260 (1972).
6. L. Heding, *Diabetologia* **7**, 10 (1971).
7. A. Andersson, *Biochem. J.* **140**, 377 (1974).
8. R. Abraham, R. Hendy and P. Grasso, *Expl. molec. Path.* **9**, 212 (1968).
9. M. E. Fedorko, J. G. Hirsch and Z. A. Cohn, *J. Cell Biol.* **38**, 392 (1968).
10. L. Fischer and M. Amherdt, *Diabetologia* **13**, 392 (1977), abstract.
11. B. Petersson, C. Hellerström and R. Gunnarsson, *Horm. Metab. Res.* **2**, 70 (1970).

12. S. L. Howell, J. C. Edwards and M. Whitfield, *Horm. Metab. Res.* **3**, 37 (1971).
13. B. P. Richardson, L. Turcot-Lemay, M. L. McDaniel and P. E. Lacy, *Lab. Invest.* **33**, 509 (1975).
14. P. A. Halban, C. B. Wollheim, B. Blondel, W. Niesor and A. E. Renold, *Endocrinology* **104**, 1096 (1979).
15. R. Zwahlen, B. P. Richardson and R. E. Hauser, *J. ultrastruct. Res.* **67**, 340 (1979).
16. R. Roskoski and S. R. Jaskunas, *Biochem. Pharmac.* **21**, 391 (1972).
17. C. F. Lefler, H. S. Lilja and D. J. Holbrook, Jr., *Biochem. Pharmac.* **22**, 715 (1973).
18. L. M. Gonasum and A. M. Potts, *Invest. Ophthal.* **13**, 107 (1974).
19. A. M. Giuffrida, J. Sjöstrand, A. Cambria, I. Serra, A. Vanella, M. Avitabile, J. Jarlstedt and J.-O. Karlsson, *Neuropharmacology* **15**, 439 (1976).
20. F. S. Parker and J. L. Irvin, *J. biol. Chem.* **199**, 897 (1952).
21. S. N. Cohen and K. L. Yielding, *J. biol. Chem.* **240**, 3123 (1965).
22. J. K. Seydel and O. Wassermann, *Biochem. Pharmac.* **25**, 2357 (1976).
23. H. G. Lawford and P. B. Garland, in *Dynamics of Energy-Transducing Membranes* (Eds. L. Ernster, R. W. Estabrook and E. Slater), p. 159. Elsevier/North-Holland Biomedical Press, Amsterdam (1974).
24. P. Köhler and R. Bachmann, *Molec. Pharmac.* **14**, 155 (1978).
25. W. J. Malaisse, A. Sener, A. Herchuelz and J. C. Hutton, *Metabolism* **28**, 373 (1979).
26. S. DiDonato, U. N. Wiesmann and N. Herschkowitz, *Biochem. Pharmac.* **26**, 7 (1977).
27. G. D. Bloom, B. Hellman, L.-Å. Idahl, J. Sehlin and I.-B. Täljedal, *Biochem. J.* **129**, 241 (1972).
28. M. Wibo and B. Poole, *J. Cell Biol.* **63**, 430 (1974).
29. Ch. DeDuve, Th. DeBassy, B. Poole, A. Tronet, P. Tulkens and F. van Hoof, *Biochem. Pharmac.* **23**, 2495 (1974).
30. S. O. Lie and B. Schofield, *Biochem. Pharmac.* **22**, 3109 (1973).
31. M. Frisk-Holmberg, J. Bergkvist, B. Domeij-Nyberg, L. Hellström and F. Jansson, *Clin. Pharmac. Ther.* **25**, 345 (1979).