Biochemical Pharmacology, Vol. 33, No. 9, pp. 1465–1470, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

EFFECTS OF CHLOROQUINE ON LIPID METABOLISM OF MOUSE PANCREATIC ISLETS

ANDERS HALLBERG,*† CHRISTIAN BERNE‡ and ARNE ANDERSSON*
Departments of Medical Cell Biology* and Internal Medicine‡, University of Uppsala, Uppsala, Sweden

(*Received* 18 *July* 1983; *accepted* 28 *November* 1983)

Abstract—Pancreatic islets accumulate the amphiphilic drug, chloroquine, which leads to a marked impairment of the insulin production of the B-cells *in vitro*. In this study the effects of the drug on islet lipid metabolism *in vitro* were investigated. It was found that exposure of islets to chloroquine (10^{-5} M) for one week induced an increase of D-[U-^{14}C]glucose incorporation into phospholipids and triacylglycerols of about two-fold and into diacylglycerols of about six-fold. Furthermore, two-dimensional thin-layer chromatography showed an increased rate of D-[U-^{14}C]glucose incorporation into all major phospholipid classes. A 17% increase of the total islet phospholipid content indicated that an accumulation of islet cell phospholipids occurred. Pulse-chase experiments with D-[U-^{14}C]glucose showed that the rate of degradation of islet phospholipids was decreased after prolonged exposure to chloroquine. No short-term effects of chloroquine upon either the *de novo* biosynthesis of islet lipids or upon phospholipid degradation could be demonstrated. The present data therefore suggest that chloroquine impairs the rate of islet lipid degradation in long-term experiments, which may be a primary lesion in the sequence of events leading to functional impairment of the B-cell.

The finding of Dencker *et al.* [1] that chloroquine, the antimalarial and antirheumatic drug, was taken up and accumulated by pancreatic islet cells initiated further investigations in which the *in vitro* uptake of the drug [2] and its effects on the insulin production [3] were studied. Besides functional impairment of islets cultured in the presence of chloroquine, morphological findings of so called 'myeloid bodies' were made [3]. Since these bodies are a characteristic of drug-induced lipidosis [4], it was of interest to investigate effects of chloroquine on the lipid metabolism of isolated pancreatic islets maintained in tissue culture.

MATERIALS AND METHODS

Chemicals. RPMI 1640 was obtained from Flow Laboratories, Irvine, Scotland, U.K. and crude collagenase (Type CLS) from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Calf thymus DNA, chloroquine diphosphate, palmitic acid, dipalmitoylglycerol, tripalmitoylglycerol, phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Phosphatidylserine (PS), lysophosphatidylcholine (LPC) and sphingomyelin (Sph) was from Koch-Light Laboratories Ltd., Colnbrook, Bucks, England, U.K. An emulsion of egg-yolk lipids was a kind gift from AB Vitrum, Stockholm, Sweden. D-[U-^{14}C]glucose was supplied by Amersham International, Amersham, England, U.K. Malachite green (C.I. 42 000) was from BDH Chemicals Ltd., Poole, England, U.K. and Tween 20 from KEBO AB, Stockholm, Sweden. Econofluor was obtained from New England Nuclear, Boston, MA, U.S.A. and Hyamine from Packard Instruments Company Inc., Downer's Grove, IL, U.S.A. Organic solvents, 2',-7'-dichlorofluorescein and inorganic salts were of analytical grade and supplied by E. Merck, Darmstadt, FRG. Deionized, double-distilled water was used in all experiments.

Animals and islet preparation. Male NMRI mice (Anticimex, Sollentuna, Sweden) weighing 25–30 g were used. After starvation overnight the animals were killed by neck-dislocation. Islets were isolated by means of collagenase digestion [5].

Islet culture. Islets were cultured free-floating [6] at 37°C in a humidified atmosphere containing 5% CO_2 in air for 5–7 days in medium RPMI 1640 (11 mM glucose) supplemented with antibiotics (benzylpenicillin, 100 U/ml and streptomycin, 0.1 mg/ml) and 10% calf serum with or without addition of 10^{-5} M chloroquine, which also was the concentration of the drug used in all other experiments described. The dose–response relationship for chloroquine effects on islet B-cell functions *in vitro* has recently been described [3]. The culture media were changed on days three and five of culture.

Incorporation of D-[U-^{14}C]*glucose*. These experiments were carried out essentially as described by Berne [7]. Cultured islets were incubated in groups of 20 in glass vials containing 100 μl of a salt solution (pH 7.4) buffered with bicarbonate [8, 9] and in some experiments supplemented with 10 mM HEPES. Glucose (2.8 or 16.7 mM) and 10 μCi of D-[U-^{14}C] glucose (spec. rad. 269.7 mCi/mmole; final spec. rad. = 31.7 and 5.3 mCi/mmole, respectively) were also added. In some experiments chloroquine was added to the incubation medium. The vials were suspended in 20 ml glass flasks containing 0.5 ml

† Correspondence to be addressed to Anders Hallberg, Department of Medical Cell Biology, Box 571, S-751 23 Uppsala, Sweden.

redistilled water and 5% CO_2 in oxygen. Incubations lasted for 120 or 180 min at 37° in a shaking water bath (about 100 strokes/min). At the end of the incubation, 0.2 ml of isotope-free medium (37°) was added to each vial and the incubation was continued for another 10 min. Islets were then washed twice in an isotope-free incubation medium and transferred to glass-tubes containing 100 μl Folch's salt solution [10]. After sonication for 20 sec in an MSE Ultrasonic Disintegrator, model 60 W (MSE, London, England, U.K.) homogenates were stored overnight under N_2 at −20°.

'Pulse-chase' experiments. In order to determine the extent of phospholipid degradation in islets, pulse-labelling with [^{14}C]-glucose for 2 hr was performed with or without the addition of chloroquine as described above. After washing twice with isotope-free medium, islets were transferred to microvials containing 0.5 ml of RPMI 1640 + 10% calf serum, with or without chloroquine. The chase period lasted for 3–30 hr under general culture conditions as described above.

Separation of major lipid classes. Two ml of chloroform–methanol (2:1 v/v) were added to each homogenate. In some of the pulse-chase experiments samples of the chase incubation media were treated in an identical way. After 2 hr 30 μl of a carrier solution containing egg-yolk phospholipids, 1,2-dipalmitoylglycerol, palmitic acid and tripalmitoylglycerol were added. Four washings were performed to remove water-soluble substances from the lipid extracts [10]. A sample (1 ml) of the lipid extracts was evaporated to dryness under a stream of N_2, redissolved in 40 μl of chloroform:methanol (2:1) and 30 μl was applied to precoated silica gel H plates (20 × 20 cm) (E. Merck, Darmstadt, F.R.G.), which had been activated for 1 hr at 120°C before use. The solvent solution contained *n*-hexane-diethyl ether–methanol–acetic acid (90:20:2:3, v/v) [11]. After chromatography the lipid spots were sprayed with 2',7'-dichlorofluorescein dissolved in ethanol (0.2 mg/ml), and identified by u.v. light. The spots corresponding to phospholipids, diacylglycerols and triacylglycerols were scraped into scintillation vials and 150 μl methanol was added to each vial in order to dissolve the lipids. Blank scrapings were treated in the same way. Scintillation counting was performed after addition of 2.5 ml Econofluor®.

Separation of phospholipid classes. Extraction was performed as described above with minor modifications. As carrier solution an extract of mouse liver lipids was used. In order to increase the total amount of labelled phospholipids to be separated by TLC, all of the washed lipid extract (about 1.6 ml) was taken to dryness under N_2. The re-dissolved lipid extract was then applied to the plate. Separation of phospholipid classes was performed with 2-dimensional TLC [12]. Prior to use, silica gel H plates (10 × 10 cm) were sprayed with 3% (w/v) $MgSO_4$ solution, air dried and activated for 1 hr at 120°. The plates were developed in the first dimension with chloroform–methanol–28% aqueous ammonia (65:25:5, v/v). After air-drying for 30 min the second dimension was developed with chloroform–acetone–methanol–acetic acid–water (5:2:1:1:0.5, v/v). Spots were visualized with iodine vapors, scraped into scintillation vials and treated as described above. A distinct separation of PS, PI, LPC, Sph, PA and PC was obtained. PE and PG were migrating as a single spot. In model experiments, identification of each phospholipid spot was obtained by development of commercial phospholipid standards in parallel with the mouse liver lipid extract.

Determination of total lipid phosphorus. Groups of 50 cultured islets were transferred to glass tubes containing 125 μl of Folch's salt solution [10]. Homogenization, lipid extraction and washing procedure was performed as described above, with the exception that no carrier solution was added. The lipid extracts were transferred to glass tubes previously washed in dichromate–sulphuric acid solution overnight. After evaporation of the solvent, total lipid phosphorus was determined colorimetrically by the method of Itaya and Ui [13] after phospholipid digestion according to Chalvardjian and Rudnicki [14]. Perchloric acid (50 μl : 70%) was added to the dried lipid extracts and digestion was performed for 15 min in a sand bath (type HS42, Gerhardt, Bonn, F.R.G.) heated to 230°. The glass tubes were then cooled to room temperature and 400 μl of double-distilled water were added to each sample, followed by 2 ml of an ammonium molybdate malachite green reagent solution. After addition of 80 μl 1.5% Tween 20, each sample was shaken and the colour developed was read at 660 nm in a Zeiss Spectrophotometer, model PMQ II. KH_2PO_4 standards containing 1–10 nmole phosphorus and blank samples were carried through the same procedure.

Determination of DNA content. Duplicate samples of 10 μl were taken from each homogenate and DNA was measured fluorimetrically by the method of Kissane and Robins [15] as modified by Hinegardner [16].

Expression of results. The specific radioactivity of the incubation media was used to calculate the incorporation rate into the lipid fractions. For that purpose triplicate 5 μl samples of the incubation medium were counted after addition of 250 μl Hyamine®, 150 μl methanol and 2.5 ml Econofluor®. Results are expressed as means ± S.E.M. For statistical analyses, Student's *t*-test for independent data was used. If not otherwise stated a two-tailed test was performed.

RESULTS

Long-term effects of chloroquine on incorporation of D-[U-^{14}C]*glucose into islet major lipid fractions.* During incubation for 2 hr in a low glucose concentration (2.8 mM) cultured control islets incorporated D-[U-^{14}C]glucose into phospholipids, diacylglycerols and triacylglycerols as seen in Fig. 1. A great majority (80–90%) of the radioactivity was found in the islet phospholipid fraction (Fig. 1a) but also the diacylglycerols (Fig. 1b) and triacylglycerols (Fig. 1c) contained some radioactivity. A high glucose concentration (16.7 mM) in the incubation medium resulted in a 4- to 7-fold increase of the incorporation rates into all three lipid fractions.

Exposure to chloroquine during the one-week culture period significantly stimulated the incorporation rates into all islet lipids at both glucose con-

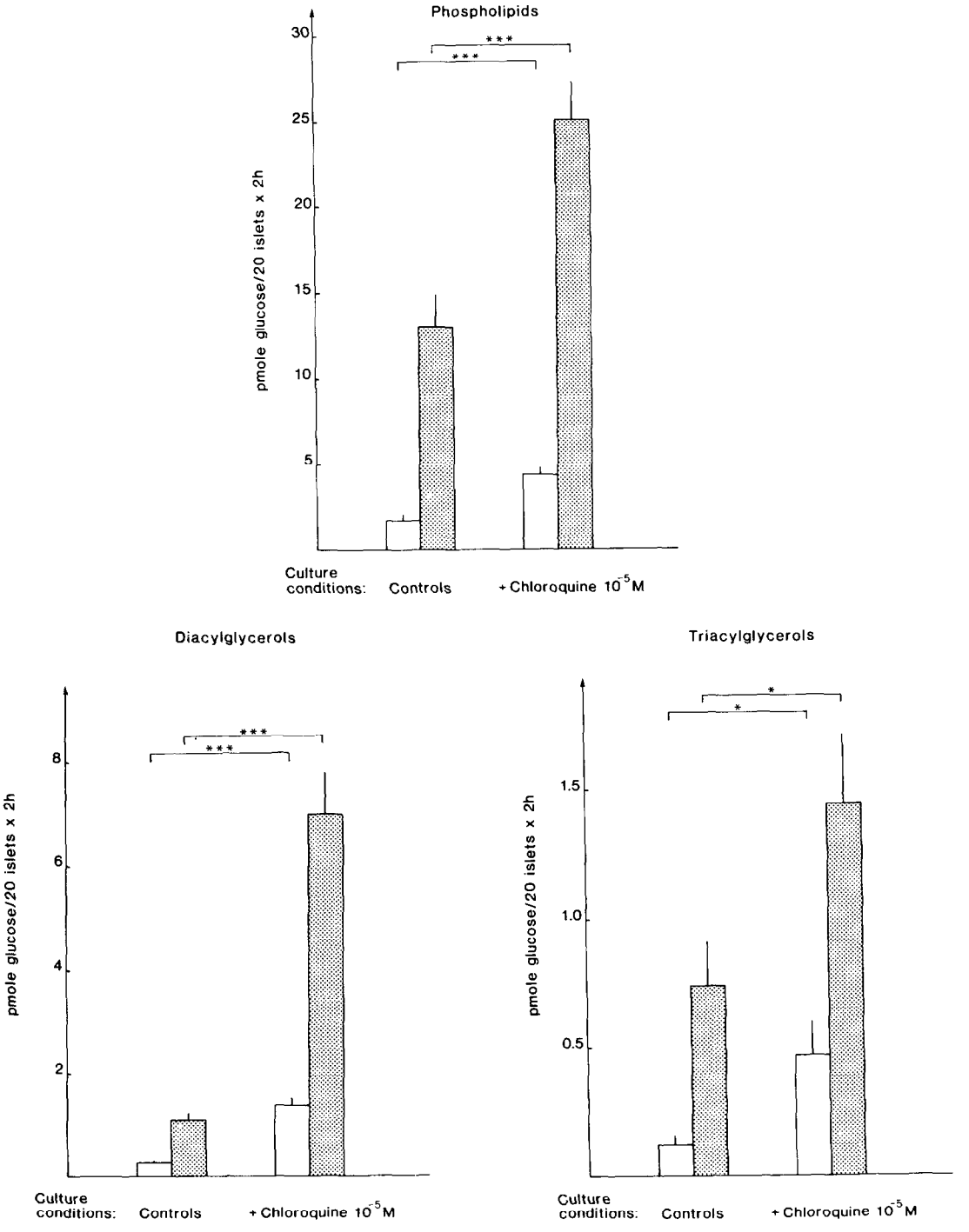

Fig. 1. Incorporation of D-[U-^{14}C]glucose in the absence of chloroquine into lipid fractions of islets, cultured for one week in the absence or presence of 10^{-5} M chloroquine. The incubation medium (Gey and Gey buffer) contained 2.8 mM (open bars) or 16.7 mM glucose (stippled bars). a; phospholipids, b; diacylglycerols, c; triacylglycerols. Each bar represents the mean of 9–10 experiments.* $P < 0.05$, ***$P < 0.001$.

centrations. At the high glucose concentration the drug-induced increment of the incorporation was most pronounced in the diacylglycerol fraction. Thus, on a paired basis the ratios between drug-exposed and control islets were 2.07 ± 0.22, 6.68 ± 1.03 and 2.35 ± 0.56 for the phospholipid, diacylglycerol and triacylglycerol fractions, respectively ($P < 0.001$ and $P < 0.01$ when diacylglycerol was tested versus phospholipids or triacylglycerol; $N = 5$).

Long-term effects of chloroquine on incorporation of D-[U-^{14}C]*glucose into islet phospholipid classes.*

Table 1. Long-term effects of chloroquine on the incorporation of D-[U-^{14}C]glucose into islet phospholipid fractions (upper part) and on the disappearance of labelled phospholipids (lower part) in cultured mouse pancreatic islets

Culture condition	'Chase'-period (hours)	Phospholipid fraction PS	PI	Sph	PC	PE+PG
RPMI 1640	—	1.6 ± 0.1 (12)	5.1 ± 0.7 (12)	n.d.	32.2 ± 3.0 (12)	8.0 ± 0.6 (12)
RPMI 1640 + chloroquine 10^{-5} M	—	2.7 ± 0.2‡ (12)	11.1 ± 0.8‡ (12)	1.8 ± 0.3 (12)	44.9 ± 3.7* (12)	13.2 ± 1.3† (12)
RPMI 1640	30	1.2 ± 0.1 (16)	1.3 ± 0.1 (16)	n.d.	11.1 ± 0.8 (16)	3.3 ± 0.4 (15)
RPMI 1640 + chloroquine 10^{-5} M	30	1.6 ± 0.2* (14)	3.4 ± 0.2‡ (14)	1.5 ± 0.2 (14)	17.7 ± 1.1‡ (14)	6.8 ± 0.6‡ (14)

After culture in the presence or absence of chloroquine, islets were incubated for 2 hr in Krebs–Ringer–bicarbonate–HEPES buffer containing 16.7 mM glucose in the presence of the isotope but in the absence of chloroquine. In the experiments of the lower part of the table the incubation was followed by a 'chase'-period in isotope-free RPMI 1640 without chloroquine. Means ± S.E.M. are given and data are expressed as pmole glucose/20 islets. Numbers of experiments are given within parentheses. n.d. = not detectable.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ when tested vs corresponding controls cultured in the absence of chloroquine.

The possibility that long-term chloroquine exposure may cause an altered incorporation of D-[U-^{14}C] glucose into some of the different islet phospholipid classes was investigated in a separate series of experiments. Using 2-dimensional TLC for separation of islet phospholipid classes, detectable amounts of radioactivity were present in PS, PI, PC and PE + PG in cultured islets after an incubation for 2 hr in the presence of D-[U-^{14}C]glucose (Table 1). The incorporation of the isotope into LPC, Sph and PA of these islets was too small to be detected. Exposure of the islets to chloroquine during the culture period significantly increased the incorporation of the isotope into PS (160%), PI (218%), PC (139%) and PE + PG (165%). Exposure to chloroquine during culture also increased the incorporation of the isotope into Sph to a level higher than blank values.

Total islet phospholipid and DNA content. In some groups of islets cultured in the presence or absence of chloroquine the total islet phospholipid content was estimated by measurements of total lipid phosphorus. In islets cultured in RPMI 1640, the total phospholipid content was 4.1 ± 0.2 μg/μg DNA (N = 10). Exposure to chloroquine during culture increased this value to 4.8 ± 0.3 μg/μg DNA (N = 10); ($P < 0.05$). No difference in the islet DNA content of the two experimental groups was observed. Thus, chloroquine-exposed islets contained 1.18 ± 0.09 and the controls 1.12 ± 0.09 μg DNA/50 islets (N = 10).

Effect of chloroquine on 'pulse-chase' labelling of islet phospholipids with D-[U-^{14}C]*glucose.* To determine the rate of phospholipid degradation in cultured islets, the radioactivity incorporated at 16.7 mM glucose into islet phospholipids was chased for another 3–30 hr in isotope-free culture medium. It was found that in islets cultured in RPMI 1640 the degradation of phospholipids progressed slowly (Fig. 2). After 6 hr, 71% and 30 hr, 57% of the incorporated radioactivity was still to be found in the phospholipid fraction. However, there was no statistically significant degradation of phospholipids even after 30 hr in islets that had been exposed to chloroquine during the culture period despite the fact that chloroquine had been omitted from the chase-medium. No ^{14}C-labelled phospholipids could be detected in the different incubation media at the end of the chase period.

In a similar series of 'pulse-chase' labelling experiments, islets were cultured in RPMI 1640 without additions and, after incubation with the isotope, the radioactivity incorporated at 16.7 mM glucose into phospholipids was chased for 30 hr in culture-medium with or without addition of chloroquine. Under these circumstances it was found that exposure to chloroquine during the chase period did not influence the islet phospholipid content. Thus, 9.37 ± 0.75 (N = 15) and 9.26 ± 0.59 (N = 15) pmole glucose/20 islets were still incorporated in the phospholipids of chloroquine exposed islets and controls after the chase period.

The disappearance of the different labelled phospholipids in islets exposed to chloroquine during culture was determined in a separate series of experiments after a 30 hr 'chase'-period following the

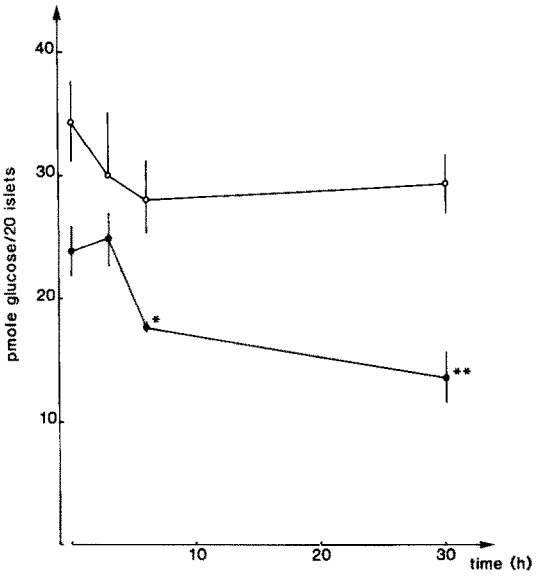

Fig. 2. Disappearance of radioactively labelled phospholipids from pulse-labelled islets cultured for one week in the absence (closed circles) or presence (open circles) of 10^{-5} M chloroquine. Pulse-labelling was performed in Gey and Gey buffer containing 16.7 mM glucose for 2 hr, followed by culture in RPMI 1640 and in the absence of chloroquine for another 3–30 hr. Each circle represents the mean of 4–10 experiments. $^{**}P < 0.01$ and $^{*}P < 0.05$ (one-tailed test) when tested vs non-chased controls.

incubation with the isotope (Table 1, lower part). In islets exposed to chloroquine, there were significantly higher levels of radioactivity still present in PS (133%), PI (262%), PC (159%) and PE + PG (206%) as compared to control islets. Furthermore, detectable amounts of radioactivity were found in Sph of chloroquine-exposed islets.

Effect of chloroquine on islet lipid biosynthesis. In an attempt to estimate a possible effect of chloroquine on the *de novo* biosynthesis of islet lipids, one-week-cultured islets (RPMI 1640) were incubated for 3 hr in the presence of D-[U-^{14}C]glucose and chloroquine. No stimulatory effect of chloroquine on the glucose incorporation into the major islet-lipid fractions was seen, as compared with islets incubated in the absence of the drug (Table 2). After the 3 hr incubation period, most of the radioactivity was still found in the phospholipid fraction. At this time point there was, however, more radioactivity incorporated into triacylglycerols than into diacylglycerols, as compared with the 2 hr incubations (cf. Figs 1b, c).

DISCUSSION

The present study shows that exposure of pancreatic islets to chloroquine for 7 days *in vitro* causes an increased incorporation of radioactive glucose into the major lipid classes and an impaired degradation rate of the islet phospholipids. As evidenced by determinations of the total islet phospholipid content this led to an accumulation of phospholipids in the chloroquine-exposed islets. In a previous study it was found that culture of islets in the presence of chloroquine caused a marked decrease of insulin biosynthesis and secretion as well as the oxidation rate of glucose [3]. Furthermore, the B-cells of the chloroquine-exposed islets contained an abundance of 'myeloid bodies', which were absent in control-cultured islets. Altogether these findings indicate that chloroquine induces a lipidosis-like condition in the islets, which has earlier been observed in several other tissues, e.g. fibroblasts [17], hepatocytes [18], leucocytes [19], pancreatic exocrine cells [19] and retinal ganglion cells [20].

The chloroquine molecule is a weak base known to accumulate within lysosomes, probably by proton trapping due to its amphiphilic properties [21]. Due to the buffering capacity of the lysosomes or, more probably, the action of a proton pump in the lysosomal membrane, the intra-lysosomal concentration of the drug may finally reach a level of thousand times higher than the surrounding medium [22]. The final steady-state concentration within lysosomes takes, however, considerably longer time to develop as compared to the rapid cytoplasmatic uptake [21]. The initial phase of chloroquine uptake into pancreatic islets is known to be rapid and still linear after 90 min [2]. The time-course for the slower, second phase accumulation of the drug into the lysosomal compartment of pancreatic islet cells is, however, to our knowledge, unknown. Nevertheless, the biphasic pattern of chloroquine uptake offers an explanation for the discrepancies in this study between the short-term and long-term effects of the drug. Thus, chloroquine exposure of the islets in short-term experiments affected neither the incorporation rate of ^{14}C-glucose into phospholipids (3 hr) nor the degradation rate of phospholipids (30 hr). On the other hand, after culture in the presence of chloroquine for a week, there was an increased rate of the [^{14}C]glucose incorporation and a delayed degradation rate of phospholipids.

A plausible explanation for the accumulation of radioactive glucose in islet phospholipids is a chloroquine-induced impairment of phospholipid degradation. Thus, the reduced degradation leads to a

Table 2. Incorporation of D-[U-^{14}C]glucose into lipid fractions of islets cultured in RPMI 1640 for one week in the absence of chloroquine and subsequently incubated at 16.7 mM glucose in Gey and Gey buffer with or without 10^{-5} M chloroquine

Additions	Phospholipids	Diacylglycerols	Triacylglycerols
None	37.0 ± 3.7 [15]	2.3 ± 0.4 [13]	4.0 ± 0.7 [13]
Chloroquine 10^{-5} M	39.5 ± 4.4 [15]	2.8 ± 0.5 [14]	4.6 ± 0.7 [14]

Incubation with the isotope was carried out for 3 hr. Means ± S.E.M. are given and data are expressed as pmole glucose/20 islets × 3 hr. Number of experiments are given within parentheses.

decreased rate of re-utilization of non-labelled precursors [23] which favours the incorporation of [^{14}C]-glucose, which is mainly incorporated as glycerol phosphate and to a minor extent as fatty acids in mouse pancreatic islets [7]. Our present findings therefore are compatible with the idea that the slow but extensive lysosomal accumulation of chloroquine is the causative factor behind the delayed degradation of phospholipids. In favour of this view, Matsuzawa and Hostetler [24] have recently demonstrated that chloroquine in mM-concentrations inhibits phospholipase A and C in isolated rat liver lysosomes by a direct interaction with the enzymes and by increasing the lysosomal pH.

In cultured control islets a high glucose concentration during the incubation period markedly increased the incorporation of [^{14}C]glucose into phospholipids, diacylglycerols and triacylglycerols as previously reported [7]. After culture in the presence of chloroquine, there was an additive effect of the drug on the incorporation rate into all three major lipid fractions. This drug-induced increment of the incorporation rate was of the same magnitude in the low and high glucose concentration, thus suggesting that the action of chloroquine is not directly linked to changes of the glucose metabolism of the islets. The reduced glucose oxidation rate of pancreatic islets cultured in 10^{-5} M chloroquine [3] raises the question of whether the increased incorporation of [^{14}C]glucose into different lipids after 5–7 days of chloroquine exposure reflects shunting of the isotope into lipids after the glycolytic conversion of glucose to glyceraldehyde phosphate and glycerol phosphate. It was, however, also found that islets exposed to 10^{-4} M chloroquine for only 90 min showed a significantly reduced oxidation rate of glucose. Since we in the present study were unable to detect an increased incorporation of [^{14}C]glucose into lipids during 3 hr of chloroquine exposure, a shunting mechanism seems unlikely. The exact location of the chloroquine-induced inhibition of glucose oxidation in pancreatic islets is, however, still unknown. It is noteworthy that the flux of glucose through glycolysis in erythrocytes has been shown to be unaffected by exposure to 1 mM chloroquine for 1 hr [25].

The accumulation of radioactive glucose after chloroquine exposure was increased not only into all major phospholipid classes but also into di- and triacylglycerols. Indeed, chloroquine increased the incorporation of [^{14}C]glucose into diacylglycerols more than into phospholipids of the long-term exposed islets. Since diacylglycerol is the immediate precursor in the biosynthesis of some phospholipids (PC, PE, PS) an inhibited degradation of these lipids could effect a feedback inhibition of their biosynthesis with a corresponding accumulation of diacylglycerol.

To what extent the derangement of the phospholipid metabolism induces the marked impairment of insulin production in the chloroquine exposed B-cells [3] is difficult to assess. To our knowledge there are no previous reports on the influence of disturbances of the islet phospholipid metabolism on the insulin production. It is, however, tempting to believe that changes in the membrane turnover of the rough endoplasmic reticulum leads to a deterioration of the intracellular insulin processing (biosynthesis, storage, release and degradation), which finally gives the severe functional impairment.

Acknowledgements—We are grateful to Margareta Engkvist for excellent technical assistance and to Kerstin Claesson and Agneta Snellman for the preparation of the manuscript. Financial support from the Swedish Medical Research Council (12X-109), the Swedish Diabetes Association and the Medical Faculty of Uppsala University is gratefully acknowledged.

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. Toxicol.* **47**, 38 (1980).
3. A. Andersson, S. Olsson and H. Tjälve, *Biochem. Pharmac.* **29**, 1729 (1980).
4. H. Lüllman, R. Lüllman-Rauch and O. Wassermann, *Crit. Rev. Toxicol.* **4**, 185 (1975).
5. S. L. Howell and K. W. Taylor, *Biochem. J.* **108**, 17 (1968).
6. A. Andersson, *Diabetologia* **14**, 397 (1978).
7. C. Berne, *Biochem. J.* **152**, 667 (1975).
8. G. O. Gey and M. K. Gey, *Am. J. Cancer* **27**, 45 (1936).
9. H. A. Krebs and K. Henseleit, *Hoppe-Seylers Z. physiol. Chem.* **210**, 33 (1932).
10. J. Folch, M. Lees and G. H. Sloane Stanley, *J. biol. Chem.* **226**, 497 (1957).
11. P. B. Garland and P. J. Randle, *Nature, Lond.* **199**, 381 (1963).
12. G. Rouser, S. Fleischer and A. Yamamoto, *Lipids* **5**, 494 (1970).
13. K. Itaya and M. Ui, *Clinica chim. Acta* **14**, 361 (1966).
14. A. Chalvardjian and E. Rudnicki, *Analyt. Biochem.* **36**, 225 (1970).
15. J. M. Kissane and E. Robins, *J. biol. Chem.* **233**, 184 (1958).
16. R. T. Hinegardner, *Analyt. Biochem.* **39**, 197 (1971).
17. S. O. Lie and B. Schofield, *Biochem. Pharmac.* **22**, 3109 (1973).
18. A. Yamamoto, S. Adachi, Y. Matsuzawa, T. Kitani, A. Hiraoka and K.-I. Seki, *Lipids* **11**, 616 (1976).
19. M. E. Fedorko, *Lab. Invest.* **18**, 27 (1968).
20. R. Abraham and R. J. Hendy, *Exp. Mol. Pathol.* **12**, 185 (1970).
21. C. de Duve, T. de Barsy, B. Poole, A. Trouet, P. Tulkens and F. Van Hoof, *Biochem. Pharmac.* **23**, 2495 (1974).
22. M. Wibo and B. Poole, *J. Cell Biol.* **63**, 430 (1974).
23. H. Van den Bosch, *Biochim. biophys. Acta* **604**, 191 (1980).
24. Y. Matsuzawa and K. Y. Hostetler, *J. biol. Chem.* **255**, 5190 (1980).
25. S. N. Kelman, S. G. Sullivan and A. Stern, *Biochem. Pharmac.* **30**, 81 (1981).