

Role of Microtubules in the Synthesis, Conversion, and Release of (Pro)Insulin

A BIOCHEMICAL AND RADIOAUTOGRAPHIC STUDY IN RAT ISLETS

F. MALAISSE-LAGAE, M. AMHERDT, M. RAVAZZOLA, A. SENER, J. C. HUTTON,
L. ORCI, and W. J. MALAISSE, *Institute of Histology and Embryology,
Geneva University Medical School, Geneva, Switzerland, and
Laboratory of Experimental Medicine, Brussels University, Brussels, Belgium*

ABSTRACT In the pancreatic B cell, microtubules are thought to be involved in the process of insulin release. Their possible participation in the sequence of events leading from the biosynthesis and conversion of proinsulin to the release of newly synthesized insulin was investigated in rat isolated islets exposed to colchicine (0.1 mM). When the islets were preincubated for 30 min with colchicine and [^3H]leucine and, thereafter, incubated for two successive periods of 90 min each, still in the presence of colchicine, the release of preformed insulin was progressively inhibited and that of newly synthesized hormone delayed. When the islets were preincubated for 120 min with colchicine, subsequently pulse-labeled with [^3H]leucine, and eventually examined by ultrastructural autoradiography, the export of newly synthesized proinsulin out of the rough endoplasmic reticulum, its transit through the Golgi complex, and its eventual packaging in secretory granules were all retarded. This situation was associated with a delayed conversion of proinsulin to insulin. Under the same experimental conditions, colchicine failed to affect the oxidation of glucose and adenylate charge in the islets. The effect of colchicine upon the release of preformed and newly synthesized insulin was not reproduced by lumicolchicine. It is concluded that colchicine interferes with the system controlling the intracellular transfer of secretory material from site of synthesis to site of release. This interference is likely to be linked to the effect of colchicine on microtubules.

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INTRODUCTION

An array of ultrastructural, biochemical, functional, cinematographic, and pathological observations indicate that, in the pancreatic B cell, a microtubular-microfilamentous system is involved in the process of insulin release, as recently reviewed (3). However, only scanty data (4–7) are so far available concerning the participation of such a microtubular-microfilamentous system in the regulation of proinsulin synthesis and conversion. This work examines the effect of colchicine in the sequence of events leading from the biosynthesis and conversion of proinsulin to the release of newly synthesized hormonal peptides (8–13).

METHODS

All experiments were performed with isolated islets removed from fully fed albino rats (14). Colchicine was purchased from Sigma Chemical Co. (St. Louis, Mo.). For the preparation of lumicolchicine (15), a 2.5-mM solution of colchicine in 95% ethanol was irradiated for 50 h with a long-wave ultraviolet lamp (Universal UV Lampe; Camag, Müttenz, Switzerland). After partial evaporation of the solvent under a stream of N_2 , the residual solution was mixed with an equal volume of H_2O and lyophilized. The absorbancy at 350 nm was reduced to a minimum of 5% of the original optical density.

Effect of colchicine and lumicolchicine upon the release of preformed and newly synthesized insulin. Groups of 70 or 35 islets each were preincubated for 30 min (min 0–30) in 1.0 ml of bicarbonate-buffered medium (16) which contained bovine albumin (5.0 mg/ml), L-[4,5- ^3H (N)]leucine (100 $\mu\text{Ci}/\text{ml}$, 1.7–2.4 μM , New England Nuclear, Boston, Mass.), glucose (16.7 mM), and, when required, either colchicine or lumicolchicine (0.1 mM). The islets were then submitted to four successive washes performed at room temperature (17). The washing medium (1.0 ml) was the same as that used for pre-

incubation except for the absence of leucine. The length of the washing procedure did not exceed 15 min. The islets were then incubated for two successive periods of 90 min each (min 30–120 and min 120–210) in 1.0 ml of medium which again contained glucose (16.7 mM) and, as required, colchicine or lumicolchicine (0.1 mM). At the end of the second incubation, the islets were sonicated (17) in 1.0 ml of the bicarbonate-buffered solution. The technique used for the measurement of insulin release and insulin content in the islets is described in detail elsewhere (16, 18). Immunoreactive insulin (IRI)-like tritiated peptides ($[^3\text{H}]\text{IRI}$) were measured in both incubation media and islet homogenates with a method identical to that reported elsewhere (4). The principle of this method is to allow insulin to react with an excess of anti-insulin serum.

Effect of colchicine on proinsulin synthesis and conversion. Groups of 25 islets each were preincubated for 120 min in 1.0 ml of a medium which contained glucose (16.7 mM) and, when required, colchicine (0.1 mM). The islets were then labeled with $[^3\text{H}]\text{leucine}$ (210 $\mu\text{Ci/ml}$; 3.6 μM) during a 10-min incubation performed in media (0.3 ml) of otherwise identical composition as those used for preincubation. The islets were washed twice at room temperature with 1.0 ml of a nonradioactive medium and, thereafter, incubated for 10, 25, 55, or 85 min in 1.0 ml of medium which contained glucose (16.7 mM), unlabeled leucine (1.0 mM), and, when required, colchicine (1.0 mM). The release of insulin was measured only during this last incubation period. After incubation, the islets were sonicated (17) at 0–4°C in 0.3 ml of acetic acid (2 M). The technique used for the separation of ^3H -peptides in islet homogenates by polyacrylamide gel chromatography was previously described (17). Fig. 1 illustrates the pattern of results obtained by such a method.

Effect of colchicine upon the radioautographic distribution of ^3H -material. The experimental protocol was comparable to that used to investigate the effect of colchicine upon proinsulin synthesis and conversion. Thus, the islets were preincubated for 120 min (40 islets/1.0 ml) in media that contained glucose (16.7 mM) with or without colchicine (0.1 mM); labeled over 5-min incubation (200 islets/1.0 ml) in the presence of $[^3\text{H}]\text{leucine}$ (125 $\mu\text{Ci/ml}$; 2.5 μM), glucose, and, when required, colchicine; washed four times at room temperature with media containing unlabeled leucine (5.0 mM); and eventually incubated for 0, 10, 25, 55, or 85 min in media (40 islets/1.0 ml) again containing glucose (16.7 mM), unlabeled leucine (0.2 mM), and, when required, colchicine (0.1 mM). After the latter incubation, the islets were fixed with 2% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) and further processed for electron microscopy. Electron microscopic radioautography was performed on thin sections of isolated islets (19). Thin sections were coated with Ilford L-4 emulsion (Ilford Ltd., Ilford, Essex, England), exposed for 5–7 wk, and subsequently developed at 18°C for 4 min with Microdol (Eastman Kodak Co., Rochester, N. Y.) developer, or for 1–2 min with Phenidone (Geigy Chemical Corp., Ardsley, N. Y.) developer (20). The distribution of silver grains was determined on electron micrographs of the first 10–14 fields of B-cell cytoplasm containing radioautographic grains (332 ± 26 per islet), according to the following five classes: rough endoplasmic reticulum (RER), including perinuclear cisternae, transition elements, and the surrounding cytoplasmic matrix; elements of the Golgi complex; secretory granules associated with the Golgi complex; secretory granules not associated

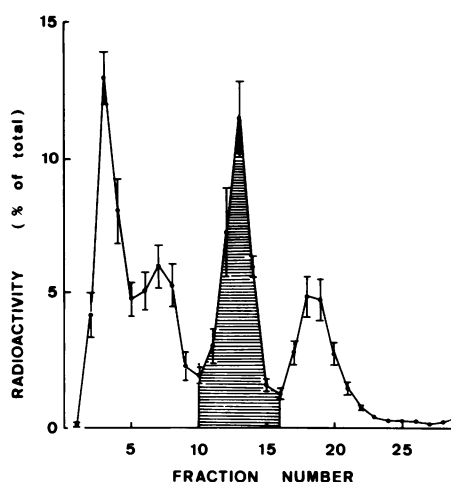


FIGURE 1 Elution pattern after polyacrylamide gel chromatography of labeled material in homogenates of islets incubated for 90 min in the presence of glucose (16.7 mM) and $[^3\text{H}]\text{leucine}$. Mean values ($\pm\text{SEM}$) for nonhormonal peptides (fraction 0–10), proinsulin (shaded area), and insulin (last peak) refer to six individual experiments are expressed in percentage of the total radioactivity eluted from the column.

with the Golgi complex; and other sites (mitochondria, nuclei, plasma membrane, lysosomes). The latter class only contained $8.5 \pm 0.6\%$ ($n = 60$) of the total amount of silver grains.

The method used to measure glucose oxidation (21) and the concentration of ATP, ADP, and AMP (22) in the islets are described elsewhere.

All results are expressed as the mean ($\pm\text{SEM}$).

RESULTS

Effect of colchicine upon the release of preformed and newly synthesized insulin

We have first investigated the extent to which a colchicine-induced alteration of the B-cell microtubular apparatus may affect the release of newly synthesized insulin. For this purpose, the islets were first exposed for 30 min to $[^3\text{H}]\text{leucine}$, then washed to remove extracellular $[^3\text{H}]\text{leucine}$, and eventually incubated for two successive periods of 90 min each in a leucine-free medium. Glucose (16.7 mM) and, when required, colchicine (0.1 mM) were present in the media throughout this procedure. The release of both insulin and $[^3\text{H}]\text{IRI}$ was measured during the two successive periods of incubation (min 30–120 and 120–210). After the second incubation, the islets were homogenized for measurement of their content of insulin, $[^3\text{H}]\text{IRI}$, and TCA-precipitable tritiated material.

In designing this experiment, we took into account the time- and dose-related effect of mitotic spindle-inhibitors upon the B-cell microtubular apparatus (23), so that the labeling of the islets occurred at a time

¹Abbreviations used in this paper: IRI, immunoreactive insulin; PCG, percentage of grains; RER, rough endoplasmic reticulum.

(min 0–30) when only a modest decrease in the number of microtubules had presumably been achieved.

Effect of colchicine upon insulin release. The release of insulin evoked by glucose during the first incubation (30th–120th min) averaged 334 ± 20 μ U/islet (Table I). It fell slightly during the second incubation (120th–210th min) to $79.4 \pm 7.4\%$ of its initial paired value. This behavior confirms previous observations (4, 5). Colchicine inhibited glucose-induced insulin secretion. Relative to its paired control value found in the absence of colchicine during the same periods of incubation, the release of insulin evoked by glucose in the presence of colchicine averaged $83.3 \pm 3.8\%$ ($P < 0.01$) and $58.9 \pm 6.7\%$ ($P < 0.001$), over the first and second period of incubation, respectively. Such a time-related increase in the inhibitory effect of colchicine is also obvious when considering the fractional rather than absolute values for insulin release (Table I).

As expected from the findings so far outlined, the residual insulin content of the islets exposed to colchicine was somewhat higher than that of the islets incubated in the absence of the mitotic spindle-inhibitor, the latter difference being significant ($P < 0.001$) judged from the increase in the fractional content ($+8.6 \pm 1.8$). The total amount of insulin released in the media and recovered in the islets was almost identical in the presence or absence of colchicine.

Effect of colchicine upon proinsulin synthesis. Colchicine reduced the amount of [3 H]leucine incorporated in the integrated amount of [3 H]IRI, i.e., that both released in the media and recovered in the islets ($-20.9 \pm 7.8\%$ by paired comparison; $P < 0.05$). The specific activity (counts per minute per microunit) of the integrated amount of insulin was consequently also reduced after exposure to colchicine ($P < 0.01$). These data, however, do not distinguish whether this reduction is related to the “antitubulin” property of colchicine or to a decreased uptake of [3 H]leucine (24), an abnormality in the intracellular compartmentation of this amino acid, or a true reduction in the B-cell biosynthetic activity. Whatever the explanation, it should be stressed that colchicine failed to affect the preferential stimulant effect of glucose upon the biosynthesis of proinsulin as distinct from that of other islet proteins (5, 17). Thus, the ratio of [3 H]IRI to total TCA-precipitable 3 H-material in the final insular homogenate was not lower after exposure to colchicine (0.25 ± 0.02) than under control conditions (0.22 ± 0.02).

Effect of colchicine upon [3 H]IRI release. To correct the above-mentioned effect of colchicine upon [3 H]leucine incorporation in insular proteins, the presentation of results dealing with the effect of the mitotic spindle-inhibitor upon release of newly synthesized peptides will be restricted to those data which illustrate

TABLE I
Effect of Colchicine upon the Release of Preformed and Newly Synthesized Insulin

Parameter	Control	Colchicine	Control	Colchicine
	μ U/islet		% of total	
Insulin				
Released from min 30 to 120	334 ± 20	276 ± 13	19.1 ± 1.1	16.4 ± 1.1
Released from min 120 to 210	258 ± 23	150 ± 17	14.5 ± 0.8	8.7 ± 0.9
Final content	$1,159 \pm 42$	$1,310 \pm 110$	66.3 ± 1.1	74.9 ± 1.4
Integrated amount	$1,751 \pm 68$	$1,735 \pm 120$	100.0	100.0
	cpm/islet		% of total	
[3 H]IRI				
Released from min 30 to 120	226 ± 20	58 ± 16	17.5 ± 2.1	5.2 ± 1.4
Released from min 120 to 210	155 ± 35	87 ± 18	11.1 ± 2.3	8.0 ± 1.6
Final content	971 ± 78	920 ± 99	71.4 ± 3.0	86.8 ± 2.3
Integrated amount	$1,352 \pm 88$	$1,064 \pm 111$	100.0	100.0
	cpm/cpm			
[3 H]IRI/ 3 H-protein				
Final content	0.22 ± 0.02	0.25 ± 0.02		
	cpm/ μ U		ratio of fractional values	
[3 H]IRI/insulin				
Released from min 30 to 120	0.67 ± 0.03	0.20 ± 0.06	0.87 ± 0.08	0.30 ± 0.08
Released from min 120 to 210	0.59 ± 0.12	0.53 ± 0.11	0.77 ± 0.16	0.87 ± 0.18
Final content	0.83 ± 0.05	0.71 ± 0.04	1.08 ± 0.04	1.14 ± 0.03
Integrated data	0.77 ± 0.03	0.61 ± 0.04	1.00	1.00

Mean values (\pm SEM) refer to 10 individual observations.

TABLE II
Effect of Colchicine upon Proinsulin Conversion

Parameter	Control	Colchicine	P
Insulin release, $\mu\text{U}/\text{min}$	1.85 ± 0.25 (12)	1.04 ± 0.15 (12)	<0.01
^3H -protein			
Total, cpm/islet	$2,066 \pm 169$ (31)	$1,601 \pm 128$ (31)	<0.05
(P + I)/total, %	65.7 ± 2.7 (31)	61.9 ± 2.0 (31)	NS
^3H proinsulin (nonconverted fraction)			
At the 10th min, %	95.8 ± 1.0 (8)	91.6 ± 1.8 (8)	NS
At the 25th min, %	82.6 ± 2.4 (8)	89.9 ± 2.1 (8)	<0.05
At the 55th min, %	23.3 ± 3.9 (8)	45.5 ± 4.9 (8)	<0.005
At the 85th min, %	8.7 ± 1.3 (7)	25.6 ± 3.7 (7)	$=0.001$

Mean values ($\pm\text{SEM}$) are shown together with the number of individual observations (in parentheses) and the statistical significance of the colchicine-induced changes. P, ^3H = protein; I, insulin.

the fate of ^3H IRI relative to the total amount of this material released by or recovered in the islets (fractional values).

The control islets released $28.5 \pm 3.0\%$ of the newly synthesized ^3H IRI over the two successive incubations. In the islets exposed to colchicine, the fractional release of ^3H IRI was markedly reduced ($P < 0.001$), amounting to only $13.2 \pm 2.3\%$ of its integrated value.

The effect of colchicine upon ^3H IRI release was further characterized by comparing, in each group, the data for the first and second incubation periods (Table I).

In the control islets, the amount of ^3H IRI released during the first and second incubation, respectively, was not significantly different, whether expressed in absolute terms (counts per minute per islet) or as a fractional release (percentage of integrated ^3H IRI). In these control islets, the trend towards a fall in ^3H IRI release during the second incubation was mainly a result of the already mentioned reduction in insulin release during the late period. Such a situation is documented by the fairly stable specific activity (counts per minute per microunit) of the ^3H insulin released during the two successive incubations in the absence of colchicine.

By contrast, in islets exposed to colchicine, the reduction in ^3H IRI release was much more pronounced during the first than during the second incubation. This phenomenon is most dramatically illustrated by the changes in specific activity of the secreted insulin. During the first incubation, such a specific activity was three times lower in colchicine-treated than in control islets ($P < 0.001$). However, during the second incubation, the specific activity of ^3H insulin released by the colchicine-treated islets tripled and, by doing so, became indistinguishable from control values.

Taken as a whole, these findings indicate that col-

chicine retards rather than diminishes the release of newly synthesized hormonal peptides.

Effect of colchicine on proinsulin synthesis and conversion

In the second series of experiments, which were designed to study the possible role of the microtubular apparatus in the conversion of proinsulin to insulin, isolated islets were preincubated for 120 min in the absence or presence of colchicine (0.1 mM), respectively. They were then exposed to ^3H leucine ($3.6 \mu\text{M}$) for 10 min (pulse-labeling period), washed to remove the extracellular tracer, and eventually incubated in

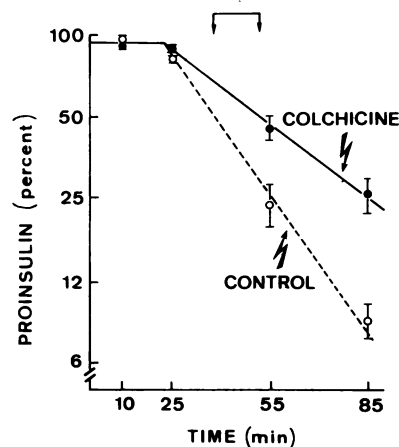
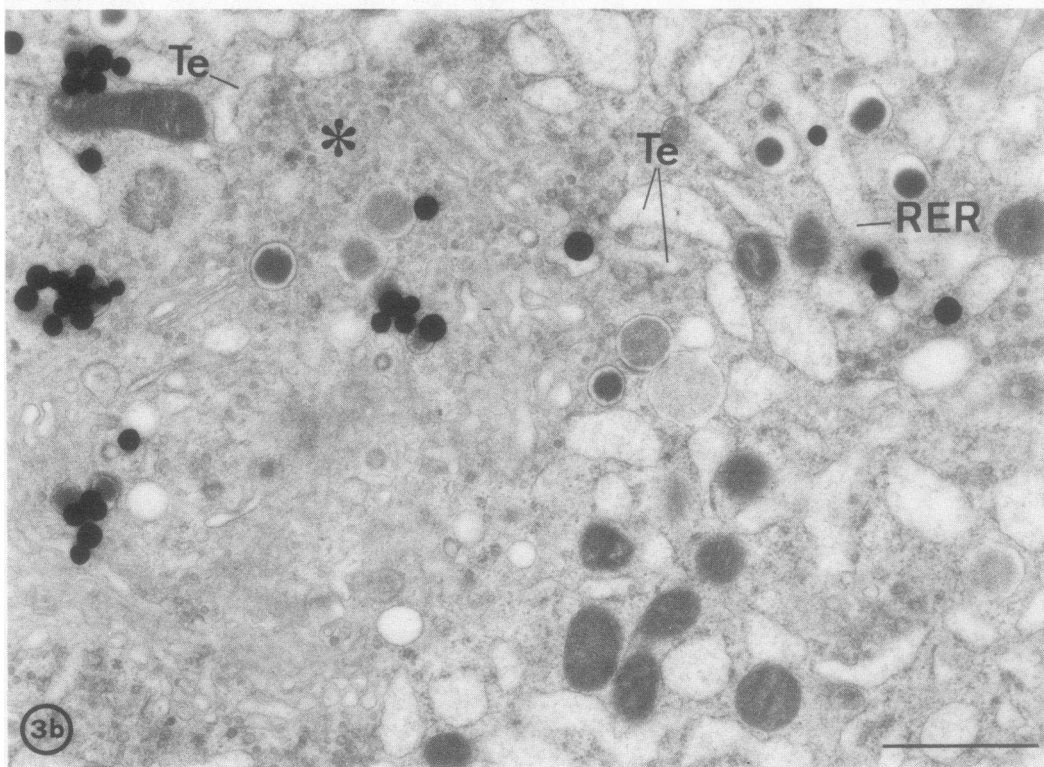
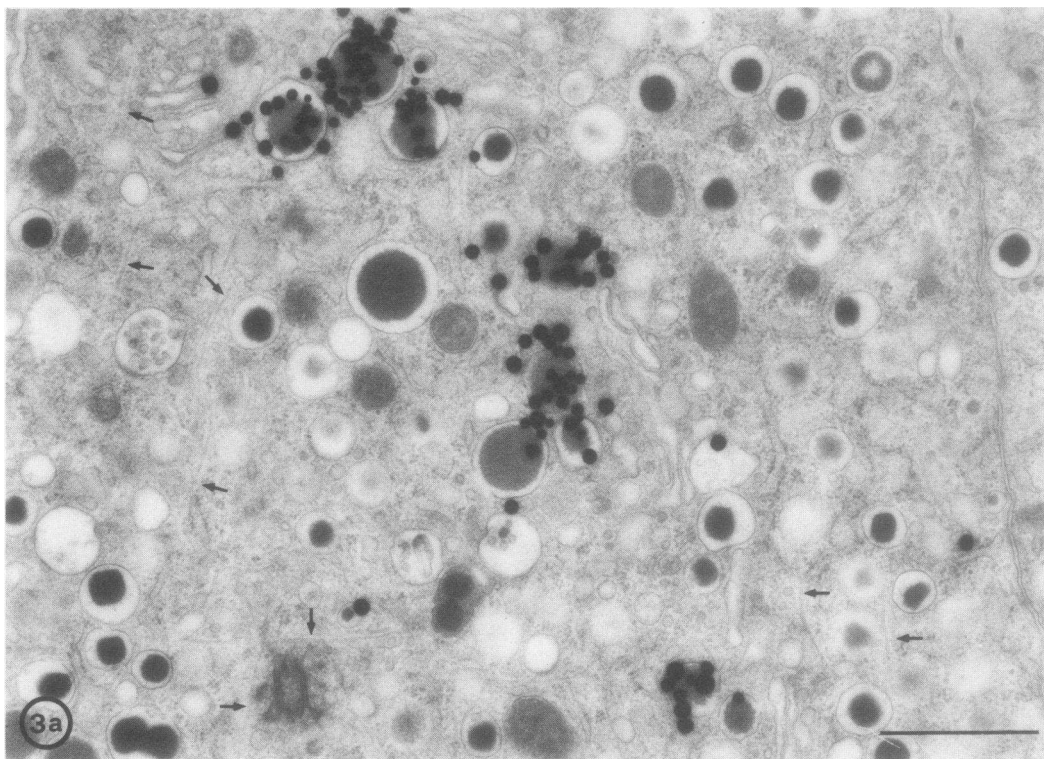


FIGURE 2 Mean values ($\pm\text{SEM}$; $n = 7-8$) for the amount of proinsulin (relative to that of proinsulin plus insulin) in control (open circles) and colchicine-treated (closed circles) islets. Also shown is the colchicine-induced delay in the time at which 50% of the proinsulin had been converted (double arrow).



the presence of unlabeled leucine (1.0 mM) for either 10, 25, 55, or 85 min (chase-incubation period). Glucose (16.7 mM) and, when required, colchicine (0.1 mM) were present in the media throughout the preincubation, pulse-labeling, and chase-incubation periods. The release of insulin was measured during the final incubation, whereas the islets were eventually homogenized in 0.3 ml of acetic acid (2.0 M) for separation of ^3H -peptides by polyacrylamide gel chromatography.

Effect of colchicine upon insulin release. The mean rate of glucose-induced insulin release in the islets incubated for 25, 55, and 85 min after labeling was significantly lower ($P < 0.01$) in the islets exposed to colchicine than the control islets (Table II). Relative to the mean control value, the rate of secretion in the islets first exposed for 120 min to colchicine averaged $56.1 \pm 7.9\%$, a value close to that found after the same length of pretreatment with colchicine in the first series of experiments, in which glucose-induced insulin release averaged, between the 120th and 210th min, $58.9 \pm 6.7\%$ of its paired control value.

Effect of colchicine upon proinsulin synthesis. The rate of [^3H]leucine incorporation in the islet proteins averaged, in the control islets, 4.45 ± 0.36 fmol/islet per min, a value close to that found, with a different technique, in the first series of experiments (4.07 ± 0.23 fmol/islet per min). The incorporation of radioactive leucine in the islet proteins was slightly but significantly reduced by colchicine, the colchicine-induced reduction averaging $22.6 \pm 6.2\%$ of the mean control value (Table II). The relative magnitude of such a reduction was almost identical to that observed for the [^3H]IRI in the first series of experiments ($20.9 \pm 7.8\%$). Once again, however, colchicine failed to affect the preferential incorporation of [^3H]leucine in hormonal peptides; the ratio of hormonal to total ^3H -peptides averaging 0.66 ± 0.03 and 0.62 ± 0.02 in the absence and presence of colchicine, respectively. Such a ratio remained fairly stable throughout the final incubation.

Effect of colchicine upon proinsulin conversion. In the control islets, the fractional amount of proinsulin converted to insulin and C-peptide progressively increased from 4.2 ± 1.0 to $91.3 \pm 1.3\%$ between the 10th and 85th min of the final incubation period (Table II). In the islets exposed to colchicine, the conversion of proinsulin was considerably delayed, $25.6 \pm 3.7\%$ of the

hormonal precursor being still present in the islets at the 85th min of incubation (Table II). When plotted in semilogarithmic coordinates, the line relating the residual relative amount of proinsulin to time suggested that, between the 25th and 85th min, the mean apparent half-life for the process of proinsulin conversion to insulin was increased by colchicine from 19 to 31 min (Fig. 2).

Effect of colchicine upon the morphology of B cells and upon the radioautographic distribution of ^3H -material

In this study, as well as in previous reports dealing with microtubule inhibitors (2, 25, 26), the most consistent alterations observed in the B cells exposed to colchicine concerned the RER and the Golgi region (Figs. 3 and 4). RER cisternae were often dilated and contained a variable amount of pale flocculent material. The Golgi region was characterized by the presence of large accumulations of microvesicles between and around Golgi cisternae. Numerous transition elements were seen contributing microvesicles. These changes were associated with a virtual absence of microtubules.

The procedure used to follow, at the ultrastructural level, the fate of ^3H -peptides in the islets was almost identical with that used for the study of proinsulin conversion (Table III), except that the exposure time to [^3H]leucine was reduced from 10 to 5 min and the concentration of unlabeled leucine in the final incubation medium increased from 1.0 to 5.0 mM. The distribution of silver grains, as determined on the electron micrographs (Figs. 3 and 4), is expressed as the percentage of grains (PCG) found over each type of organelle(s).

RER. Throughout the 90-min period of observation, more radioactivity was found in the RER of colchicine-treated than control B cells (Fig. 5). The integrated mean value (5th–90th min) averaged 35.9 ± 2.1 PCG, after exposure to colchicine and 27.1 ± 1.8 PCG in control islets, respectively ($n = 30$; $P < 0.005$). Between the 5th and 30th min, the amount of radioactivity located in the RER progressively fell from 37.8 ± 3.8 to 19.7 ± 2.8 PCG in the control islets, and from 47.3 ± 4.9 to 32.2 ± 6.2 PCG in colchicine-treated islets ($n = 6$). When corrected for an apparent phenomenon of recirculation of ^3H -material in the RER, the half-life for

FIGURE 3 Radioautographies ($\times 21,000$) of B cells. Emulsion developed with Phenidone. (a) Control B cell, preincubated for 2 h, pulse-labeled with [^3H]leucine for 5 min, and further incubated for 25 min at 16.7 mM glucose. The majority of radioautographic grains overlie the Golgi area, where they are seen mostly accumulated over maturing secretory granules. The arrows indicate microtubules. (b) B cell: same protocol as in a, except for the continuous presence of colchicine (0.1 mM). Radioautographic grains are found in the Golgi region as well as over dilated elements of the RER. The asterisk indicates accumulation of microvesicles. Te, transition elements of the RER. The bars indicate $1 \mu\text{m}$.

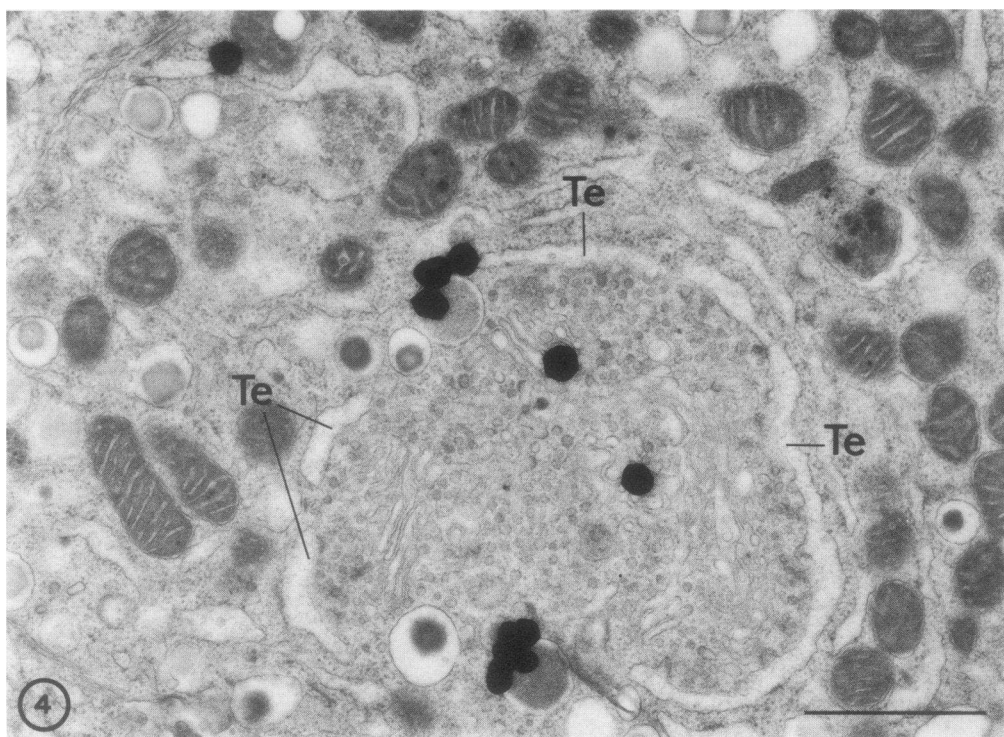


FIGURE 4 Radioautography ($\times 24,000$) of a B cell. Emulsion developed with Phenidone. B cell exposed to colchicine and examined 85 min after the pulse-labeling. Radioautographic grains are predominantly located in the Golgi region, which appears crowded by microvesicles and almost completely surrounded by transition elements (Te). The bar indicates $1\ \mu\text{m}$.

the export of radioactivity out of the RER was increased by colchicine from a control value of 9.2 to 19.8 min (Fig. 5, inset).

During the initial part of the chase period, the colchicine-induced retention of radioactivity at the RER level was matched by a delayed access of radioactivity to the Golgi complex (Fig. 6). Indeed, during this initial period, the total amount of radioactivity (PCG) located in both the RER and Golgi complex was not significantly different in the colchicine-treated and control groups. For the two groups as a whole, it averaged 83.3 ± 2.1 and 72.7 ± 2.3 PCG ($n = 12$) at the 5th and 10th min, respectively (Fig. 6a). However, the major fraction of this radioactive material had already migrated into the Golgi complex of control islets, while being still retained in the RER of colchicine-treated B cells (Fig. 6b).

Golgi complex. During the initial period (5th–15th min), significantly more radioactive material had reached the Golgi complex of control as distinct from colchicine-treated islets (Fig. 7). At a later time (60th–90th min) the opposite picture was observed, the Golgi complex of colchicine-treated islets containing significantly more radioactivity than that of control islets.

Linear extrapolation between mean measurements

performed at each time interval indicated that the integrated amount of grains transiting through the Golgi complex (as judged by planimetry) amounted to almost the same value in control (2,148 PCG/90 min) and colchicine-treated B cells (2,224 PCG/90 min). In the control and colchicine-treated islets, respectively, one-half of the latter amounts of grains had reached the Golgi complex 26.3 and 37.1 min after the onset of exposure to $[^3\text{H}]$ leucine. Thus, as judged by this criterion, colchicine delayed the transit of grains through the Golgi complex by 10.8 min, a value almost identical to that found for the colchicine-induced delay in the export of grains out of the RER (10.6 min). When the same mode of calculation was used for the data relative to the conversion of proinsulin (Table II), the colchicine-induced delay in the time at which one-half of the proinsulin had been converted to insulin and C-peptide amounted to 10.5 min. The close similarity between these values strongly suggests that the retardation in both the appearance of grains at the level of the Golgi complex and the conversion of proinsulin are two tightly interconnected phenomena. Moreover, because one-half of the proinsulin is converted at a later time than that required for the transit of one-half of the grains through the Golgi complex, the delay in the conversion

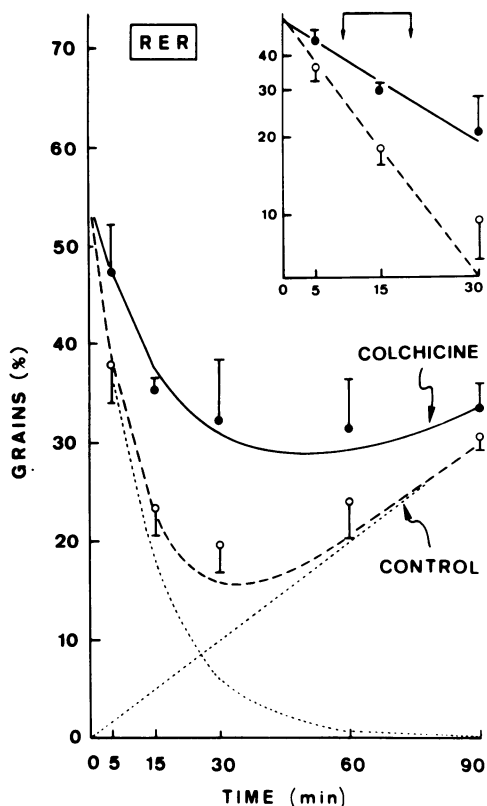


FIGURE 5 Mean values (\pm SEM; $n = 6$ in each case) for the relative radioactive content (PCG) of the RER in control (open circles) and colchicine-treated (closed circles) islets. The two upper curves (solid and dashed lines) correspond to the following equation: $PCG = (55 \cdot e^{-Kt}) + (t/3)$, in which t is the time (min) and K a constant amounting to 0.075 and 0.035 in control and colchicine-treated islets, respectively. The lower dotted lines refer to each term of the equation (in control islets). The inset illustrates the colchicine-induced change (double arrow) in the half-life for the export of grains out of the RER. Throughout the 90 min of observation, the theoretical values (calculated from the above-mentioned equation) were within the confidence interval of the observed experimental values (coefficient of correlation between theoretical and mean experimental values: 0.987; paired difference: -0.8 ± 0.6 PCG; $n = 10$).

of proinsulin is probably secondary to the abnormal transit of newly synthesized proinsulin from the RER to the Golgi complex.

Secretory granules. Except for a much later half-transit time (beyond the 45th min), the pattern seen in the β -granules located in the Golgi area (presumed immature granules) was reminiscent of that found in the Golgi complex itself (Fig. 8a). Thus, on one hand, colchicine retarded the access of radioactive material to these presumably immature granules. Indeed, between the 5th and 30th min, the rate at which the grains accumulated in such granules averaged 1.30 ± 0.05 PCG/min in control islets, as distinct from 0.80 ± 0.22

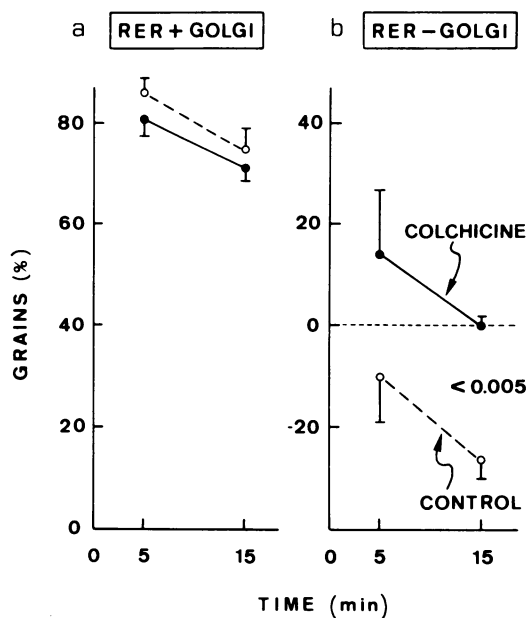


FIGURE 6 Mean values (\pm SEM; $n = 6$ in each case) for the relative radioactive content (PCG) of (a) both the RER and Golgi complex and (b) for the difference in radioactivity between RER and Golgi complex, as measured in control (open circles) and colchicine-treated (closed circles) islets. Also shown is the significance (as tested by covariance analysis) of the colchicine-induced change in elevation of the two lines drawn (b).

PCG/min in colchicine-treated islets. On the other hand, at later times (60th and 90th min), the granules located in the Golgi area contained more radioactivity in colchicine-treated as distinct from control islets; such a difference being highly significant ($P < 0.001$) at the end of the period of observation (90th min).

Over the first 30 min of incubation, very few grains (5.5 ± 0.7 PCG; $n = 36$) were seen associated with "mature" secretory granules (i.e., granules located outside the Golgi area). A significant increase was only observed at the 60th and 90th min (Fig. 8b). Over this late period, the mature β -granules contained 39.3 ± 3.0 PCG in control islets, as distinct from only 13.1 ± 2.3 PCG ($n = 12$ in both cases) in colchicine-treated B cells. This difference is highly significant ($P < 0.001$). Even when the data obtained at the 90th min in both immature and mature granules were pooled, the radioactive content of these organelles in colchicine-treated islets (35.4 ± 3.6 PCG) was still significantly lower ($P < 0.02$) than that found in control islets (52.9 ± 4.3 PCG). This situation was accounted for by the retention of radioactivity in the organelles involved in the early steps of the biosynthetic process; i.e., the RER and Golgi complex which, at the 90th min, contained 52.1 ± 3.5 and 36.1 ± 1.8 PCG ($n = 6$, $P < 0.005$) in colchicine-treated and control B cells, respectively.

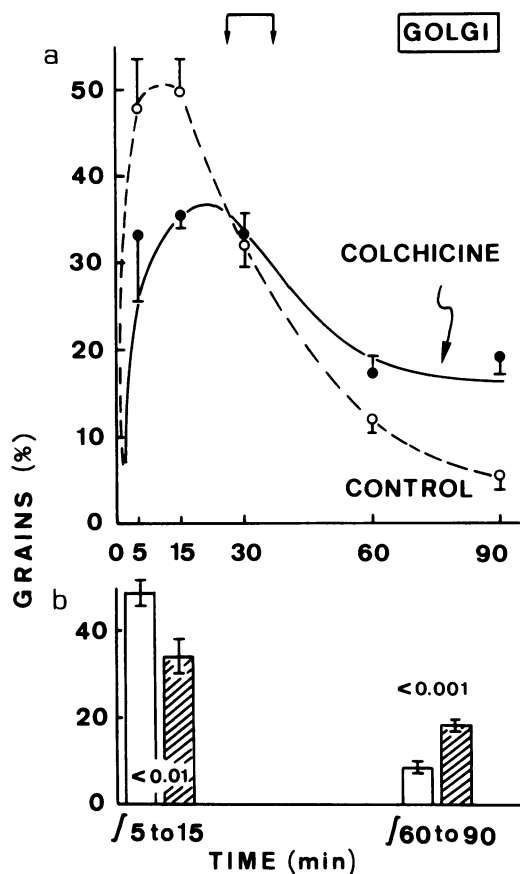


FIGURE 7 (a) Mean values (\pm SEM; $n = 6$ in each case) for the relative radioactive content (PCG) of the Golgi complex in control (open circles) and colchicine-treated (closed circles) islets. The double arrow illustrates the colchicine-induced retardation in the half-transit time of grains through the Golgi complex. (b) Mean values (\pm SEM; $n = 12$ in each case) for pooled data (f) collected from the 5th to 15th and from the 60th to 90th min in control (open columns) and colchicine-treated (hatched columns) islets. Also shown is the statistical significance of the colchicine-induced changes in radioactive content.

Effect of colchicine upon metabolic parameters

Several parameters of biosynthetic activity, such as the rate of proinsulin synthesis and the translocation of proinsulin from the RER to the Golgi complex, are influenced by the metabolism of glucose and availability of energy in islet cells (17, 27). The effect of colchicine upon such parameters could conceivably be a result of an alteration in glucose metabolism and/or energy availability, rather than being the consequence of the specific effect of colchicine upon the microtubular system. To explore the latter hypothesis, the oxidation of [14 C-U]glucose and the concentration of adenine nucleotides were measured in islets incubated in the

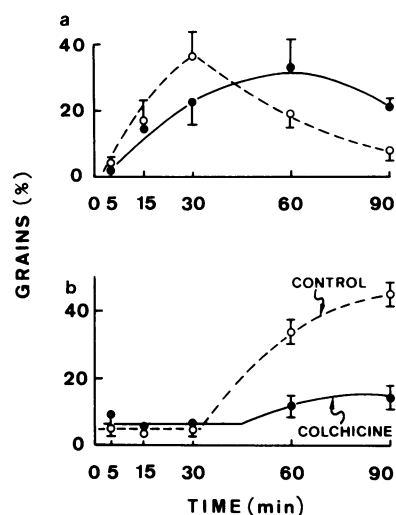


FIGURE 8 Mean values (\pm SEM; $n = 6$ in each case) for the relative radioactive content (PCG) of β -granules either (a) associated with the Golgi complex or (b) located outside the Golgi area, as measured in control (open circles) and colchicine-treated (closed circles) islets.

absence or presence of colchicine. The experimental design was comparable to that outlined in Results.

After a 120-min preincubation in the presence of unlabeled glucose (16.7 mM), the rate of glucose oxidation averaged 55.2 ± 5.9 pmol/islet ($n = 10$) over the ensuing 90 min of incubation also performed in the presence of glucose 16.7 mM. When colchicine (1.0 M, instead of the 0.1-mM concentration used in all other experiments) was present in the media during both the preincubation and incubation periods, the rate of glucose oxidation recorded during the final 90-min incubation was not significantly modified, averaging 51.4 ± 3.5 pmol/islet ($n = 9$).

As shown in Table III, colchicine (0.1 mM) also failed to affect the concentration of ATP, ADP, and AMP in the islets exposed for 120 or 210 min to the drug. By pooling the data obtained at the onset and end of the final 90-min incubation, the mean total amount of adenine nucleotides averaged 7.88 ± 0.17 and 7.88 ± 0.30 pmol/islet in control and colchicine-treated islets, respectively. Relative to such a total, the amount of ATP represented 67.9 ± 2.1 and $66.1 \pm 1.9\%$, that of ADP 25.0 ± 2.4 and $26.1 \pm 1.9\%$, and that of AMP 7.1 ± 2.4 and $7.8 \pm 2.4\%$ in the control and colchicine-treated islets, respectively. The adenylate charge averaged 0.803 ± 0.019 and 0.792 ± 0.020 in the control and colchicine-treated islets ($n = 20$ in all cases).

We were thus unable to detect any significant effect of colchicine upon metabolic parameters. The effect of colchicine upon biosynthetic activity does not seem to be attributable, therefore, to an abnormality in either glucose metabolism or energy availability.

TABLE III
Effect of Colchicine upon the Concentration of Adenine Nucleotides in Islets

Time, min	120	210	120	210
Glucose, mM	16.7	16.7	16.7	16.7
Colchicine, mM	—	—	0.1	0.1
ATP, pmol/islet	5.78±0.24	4.91±0.38	5.24±0.36	5.27±0.43
ADP, pmol/islet	1.94±0.27	2.03±0.30	2.15±0.24	1.93±0.20
AMP, pmol/islet	0.52±0.25	0.57±0.29	0.62±0.30	0.56±0.21
Total, pmol/islet	8.24±0.39	7.51±0.36	8.01±0.42	7.76±0.45
Adenylate charge	0.823±0.025	0.784±0.028	0.787±0.031	0.796±0.025

Mean values (±SEM) refer to 10 individual observations. Also shown is the time of exposure to glucose and colchicine.

Effect of lumicolchicine upon the release of preformed and newly synthesized insulin

A last series of experiments was performed in the presence of lumicolchicine (0.1 mM) according to the same protocol as that used for the experiments summarized in Table I, except that groups of 35 instead of 70 islets each were used throughout the procedure. As shown in Table IV, lumicolchicine failed to affect the release of insulin. It also failed to reproduce the effect of colchicine to delay the release of [³H]IRI. Thus, relative to the paired value found during the first incubation (min 30–120), the specific activity of the immunoreactive material released during the second in-

cubation (min 120–210) averaged 78.9±5.0 and 77.3±7.1% in control and lumicolchicine-treated islets, respectively. This behavior contrasts with the secondary rise in the specific activity of insulin secreted by colchicine-treated islets (Table I). The sole parameter which appeared slightly affected by lumicolchicine was the ratio of [³H]IRI:TCA-precipitable ³H-peptides in the final homogenate, this ratio being somewhat higher in the lumicolchicine-treated than control islets. The latter situation resulted from both a moderate decrease in the [³H]leucine content of TCA-precipitable peptides (54.3±7.5 vs. a control value of 58.3±8.0 fmol/islet) and a moderate increase in the [³H]IRI content (854±115 vs. a control value of 726±128 cpm/islet)

TABLE IV
Effect of Lumicolchicine upon the Release of Preformed and Newly Synthesized Insulin

Parameter	Control	Lumi- colchicine	Control	Lumi- colchicine
	$\mu\text{U/islet}$		% of total	
Insulin				
Released from min 30 to 120	320±9	328±11	18.3±1.5	18.4±0.5
Released from min 120 to 210	290±28	298±22	16.3±1.8	18.8±0.5
Final content	1,202±120	1,127±68	65.5±3.0	62.7±1.0
Integrated amount	1,811±124	1,753±88	100.0	100.0
	cpm/islet		% of total	
[³ H]IRI				
Released from min 30 to 120	196±32	221±20	26.2±1.6	26.5±1.5
Released from min 120 to 210	147±24	158±26	18.9±2.1	17.8±1.3
Final content	419±83	475±80	55.0±3.3	55.7±1.8
Integrated amount	762±128	854±115	100.0	100.0
	cpm/cpm		ratio of fractional values	
[³ H]IRI/ ³ H-protein				
Final content	0.12±0.01	0.15±0.01		
	$\text{cpm}/\mu\text{U}$		ratio of fractional values	
[³ H]IRI/insulin				
Released from min 30 to 120	0.60±0.09	0.68±0.07	1.46±0.11	1.32±0.09
Released from min 120 to 210	0.51±0.08	0.54±0.09	1.19±0.09	0.94±0.05
Final content	0.43±0.10	0.51±0.10	0.84±0.05	0.93±0.05
Integrated data	0.47±0.10	0.52±0.10		

Mean values (±SEM) refer to 10 individual observations.

of lumicolchicine-treated islets, none of these differences achieving statistical significance. Lumicolchicine-treated islets displayed a normal ultrastructural appearance. No obvious alteration in the frequency and structure of microtubules was observed after 60 or 120 min of lumicolchicine treatment. After the longest period of treatment (210 min) however, the frequency of microtubules was reduced; this can be a result of a slight contamination of lumicolchicine preparation by unconverted colchicine as observed by optical absorbency. Because lumicolchicine is thought not to react with tubulin (28), the comparison of the data illustrated in Tables I and IV affords further support to the concept that the major effects of colchicine upon islet function are primarily a result of an alteration of the B-cell microtubular system.

DISCUSSION

10 years ago, Lacy et al. (29) postulated that a microtubular-microfilamentous system controls the intracellular migration and extrusion of secretory granules in the pancreatic B cell. Subsequent ultrastructural and functional studies led us to postulate that the biphasic secretory response evoked by glucose in the endocrine pancreas corresponds to the summation of two components: an early secretory peak, representing the release of granules initially located in close vicinity to the cell web-plasma membrane complex, and a late component, representing, in part at least, the release of granules which have first to migrate along oriented microtubular pathways before being extruded in the interstitial milieu (23, 30).

The influence of colchicine upon the release of [^3H]IRI is consistent with such a model. On the basis of data similar to those obtained during the first incubation (30th–120th min), it was recently reported that colchicine preferentially inhibits the release of newly synthesized insulin (7). Our findings indicate that, in fact, colchicine solely retards the release of newly synthesized insulin. In other words, colchicine, by disrupting the microtubules, seems to delay more severely the release of those granules which have to travel from their site of formation in the cytocenter to the plasma membrane, than it affects the release of preformed granules conceivably stored in an area closer to their exocytotic site. At the extreme, and as already documented previously (30), the release of granules located in the immediate vicinity of the cell boundary (31) may entirely escape the colchicine-induced inhibitory effect.

The present data on the influence of colchicine upon the fate of [^3H]leucine at the ultrastructural level suggest that the microtubular apparatus also participates in the translocation of proinsulin from the RER to the Golgi complex. According to previous reports,

this energy-dependent process (27, 32) is mediated by microvesicles budding out of the RER and migrating towards the convex pole of the Golgi complex (2, 33). The integrity of microtubules, relatively numerous in this area of the B cell (23), may well be required for maintaining locally a normal rate of microvesicular traffic. Obviously, colchicine, by slowing down such a movement, will retard the transit of newly synthesized material through the Golgi complex and cause a subsequent delay in its access to secretory granules (Figs. 7 and 8).

Comparison between autoradiographic data and those relative to the conversion of proinsulin establishes that the conversion of the hormonal precursor is initiated at or shortly beyond the level of the Golgi complex and further proceeds in secretory granules. Incidentally, the half-life for the conversion of proinsulin in control islets (19 min) appears shorter than that reported by other authors (12, 13). However, the calculation used here takes into account the presumed time of onset of the conversion process. Moreover, in none of the previous investigations had the islets been stimulated with glucose for 120 min before exposure to [^3H]leucine.

The effect of colchicine upon most of the functional parameters was best defined as a retardation rather than mere inhibition. A dramatic illustration of this situation is found after 150 min of exposure to colchicine, when the secretion of preformed insulin is maximally reduced while that of newly synthesized hormone is apparently normalized. Such a dissociated behaviour clearly indicates that the effect of colchicine cannot be solely ascribed to nonspecific alteration of cellular events directly controlling the exocytotic process itself. Conversely, the failure of colchicine to abolish hormone release, despite the extensive disruption of the microtubular apparatus known to occur under our experimental conditions, suggests that microtubules are not indispensable for hormone release to occur. Hence, it is unlikely that the microtubular apparatus is the only element involved in the translocation of the secretory product from one cellular site to another.

In summary, colchicine causes a time-related decrease in the number of microtubules accompanied by a retardation of the export of proinsulin out of the RER, its transit through the Golgi complex, its subsequent conversion to insulin, and the eventual packaging of newly synthesized peptide in secretory granules. Independently, at least in part, of such effects, colchicine also retards the release of newly synthesized insulin, as it affects that of preformed hormone. A comparable situation was observed in the parathyroid gland (34, 35), exocrine pancreas (36), and chondrocytes (37). In several secreting cells, therefore, the microtubules may represent an important component controlling the

various translocations of secretory material from its site of synthesis to that of its release.

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