[^{14}C]leucine incorporation into proteins of pancreatic juice in rats fed soya-bean flour*

BY U. R. FÖLSCH† AND K. G. WORMSLEY

Department of Therapeutics, University of Dundee, Ninewells Hospital, Dundee DD2 1UB

(*Received* 5 *January* 1976 – *Accepted* 29 *March* 1976)

1. The effect of a diet containing a trypsin inhibitor on the incorporation of radioactively labelled leucine into the pancreatic proteins secreted during stimulation with cholecystokinin-pancreozymin (CCK) was studied in rats.

2. The total output of protein was significantly greater in the rats given raw soya-bean flour (RSF) compared with those given heat-inactivated soya-bean flour (HSF) (controls) in response to the sub- and supramaximal stimulation with CCK, but similar responses were obtained to maximal stimulation with CCK. Total protein output decreased continuously with time after reaching peak values at 90–120 min after the start of stimulation with CCK.

3. The total output of radioactively labelled protein in RSF-fed rats was not different from that of the controls with sub- and supramaximal dose rats of CCK, but was significantly lower than that of the controls in response to the dose rate of CCK which produced maximal rates of pancreatic secretion.

4. The specific activity of radioactively labelled protein increased continuously, while the output attained a constant rate during stimulation with all doses of CCK.

5. We concluded that feeding the trypsin inhibitor-containing diet led to increased secretion of stored pancreatic protein, while secretion of newly synthesized protein was not altered. During the course of prolonged stimulation with CCK, irrespective of diet, there was increasing secretion of the newly synthesized protein compared with the pre-existing stored proteins of the pancreas, but it was unable to compensate for the decreased secretion of pre-formed protein.

In a previous study it was found that after feeding rats for 20 d with active trypsin inhibitor in the form of a diet containing raw soya-bean flour (RSF), an increase in the number of zymogen granules in the pancreatic acinar cells was the only significant ultrastructural difference from rats given a control diet of heat-inactivated soya-bean flour (HSF) (Fölsch, Winkler & Wormsley, 1974*a*, *b*). It was also noted that the output of pancreatic enzymes in rats given RSF was greater than in controls given HSF in response to sub- and supramaximal doses of cholecystokinin-pancreozymin (CCK), but not to maximal doses (Fölsch *et al.* 1974*a*; Fölsch & Wormsley, 1974).

The present study was undertaken to determine the effect of soya-bean trypsin inhibitor on the secretion of newly synthesized pancreatic protein by estimating the incorporation of radioactively labelled leucine into proteins of pancreatic juice in rats which had been fed for 20 d on RSF or HSF.

* Presented at the 7th Symposium of the European Pancreatic Club, 1974.

† Present address: Division of Gastroenterology and Metabolism, Department of Medicine, University of Göttingen, Göttingen, W. Germany.

Table 1. *Composition of experimental diet containing either raw soya-bean flour or heated (steam-treated) soya-bean flour*

Main components of soya-bean flour (g/kg)	
Moisture	70
Protein (nitrogen × 6·25)	400
Fat	190
Phosphatides (as lecithin)	20
Ash	46
Fibre	18
Calcium	2
Phosphorus	6
Sugars (as sucrose)	60
Other carbohydrates (by difference)	180–190
'Overtails' on a 200 mesh sieve	30
Mineral and trace elements (g/kg) (added at 26·9 g/kg diet)	
$CaHPO_4$	735
K_2HPO_4	81
K_2SO_4	68
NaCl	30·6
$CaCO_4$	21
Na_2HPO_4	21·4
MgO	25
Trace element mixture*	18·0
Vitamins (/kg diet)	
Retinyl acetate† (μg)	909
Cholecalciferol (μg)	25
DL-α-tocopheryl acetate† (mg)	75
Menaphthone (mg)	1
Thiamin hydrochloride (mg)	10
Nicotinic acid (mg)	60
Riboflavin (mg)	10
Ca D-pantothenate (mg)	40
Folic acid (mg)	5
Biotin (mg)	1
Pyridoxine hydrochloride (mg)	10
Cyanocobalamin (μg)	50
Choline bitartrate (g)	1–2
Ascorbic acid (as antioxidant) (mg)	75

* Contained: KI 7·2 mg, ferric citrate 5·6 g, $ZnCO_3$ 810 mg, $MnCO_3$ 4·2 g, $CuCO_3$ 300 mg, citric acid 7·1 g.

† Added in gelatin beadlets.

MATERIALS AND METHODS

Animals and diet. Male Wistar rats (280–360 g) were used in all studies. The rats were fed for 20 d on either RSF or HSF (Soya Foods, Central House, Cambridge Road, Barking, Essex). HSF was used as the control diet because the heat-labile inhibitor had been inactivated by steam treatment. RSF and HSF were supplemented with vitamins, minerals and trace elements (I. Spencer and Co. Ltd, Wellheads Road, Farburn Industrial Estate, Dyce, Aberdeen) (Table 1) as recommended (Bernhardt & Tomarelli, 1966; Porter, 1963; Warner, 1962).

Experimental procedures. The rats were fasted for 40 h before the start of each experiment and were then anaesthetized with urethane (1·5 g/kg body-weight, intramuscularly). In order to permit collection of samples of pancreatic juice for 30 min periods, a polyethylene tube (i.d. 2 mm) was introduced into the duodenum through

an incision in the pylorus, which was tied off around the tube. A second tube was inserted into the duodenum 45 mm distally and the duodenum was ligated. The duodenum was perfused with a solution of sodium chloride (0·15 mol/l) at a temperature of 30° and at a flow-rate of 7·5 ml/15 min. The perfusate was collected in ice (Fölsch & Wormsley, 1973).

A priming dose of 10 μCi L[1-^{14}C]leucine (The Radiochemical Centre, Amersham, Bucks.) was injected according to the method of Gan & Jeffay (1967). A continuous infusion of 1 μCi L-[1-^{14}C]leucine/h was then started and continued for 5 h. After collecting the 'basal' secretion for 60 min, the rats were given a continuous intravenous infusion into the jugular vein, containing 7·5, 60 or 240 IU CCK/kg body-weight per h combined with 0·5 CU secretin/kg body-weight per h in a solution of NaCl (0·15 mol/l) at a rate of 1·65 ml/h for 4 h. The hormones were purchased from the GIH Laboratory, Karolinska Institute, Stockholm, Sweden. The three dose combinations provided respectively submaximal, maximal and supramaximal stimulation of the pancreas of the rats (Fölsch & Wormsley, 1973). Only one dose combination was used in each animal and each dose combination was studied in three rats.

Analytical procedures. The total amount of radioactivity in the samples and radioactivity after precipitation of the proteins with trichloracetic acid solution (TCA) (100 g/l) was measured in a liquid-scintillation counter (Packard Tricarb 2425; Packard Instrument Ltd, Caversham, Berks.) using glass vials filled with Insta-Gel (Packard Instrument Ltd). The protein content of the pancreatic juice was determined according to the method of Lowry, Rosebrough, Farr & Randall (1951).

The results were expressed as total amount of radioactively labelled, TCA-precipitable protein (disintegrations/min per 30 min per kg body-weight) and as specific activity (disintegrations/min per 30 min per mg protein), and were analysed statistically using the *t* test for unpaired values.

RESULTS

The total output of protein was significantly greater in RSF-fed rats than in HSF-fed animals in response to sub- ($P < 0·05$) and supramaximal ($P < 0·05$) doses of CCK but was not significantly different in response to maximal doses of CCK (Fig. 1). The specific activity of labelled proteins was significantly greater in the HSF-fed rats than in the RSF-fed animals with all dose combinations and was highest with the low dose combination (Fig. 1). The total output of newly synthesized proteins (disintegrations/min per 4 h per kg body-weight) increased significantly in all rats as the hormonal stimulation was increased from submaximal to maximal (Figs. 1 and 2). The values for total output of radioactively labelled proteins were not significantly different with sub- and supramaximal rates of hormonal stimulation, but for the group receiving maximal stimulant doses of CCK, the output of radioactively labelled proteins was significantly lower in all RSF-fed rats than in HSF-fed animals ($P < 0·01$) (Fig. 2).

While the over-all peak rates of secretion of pancreatic proteins in response to all

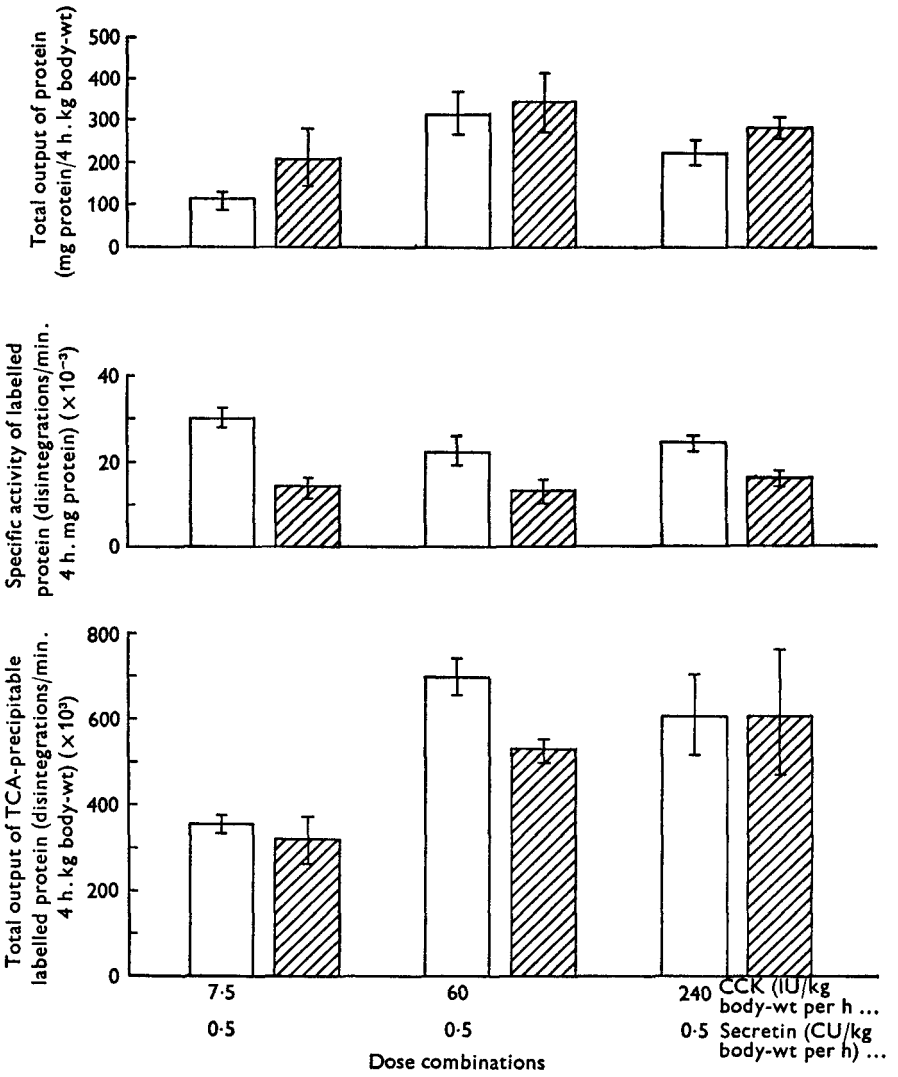

Fig. 1. Total output of pancreatic protein (mg protein/4 h per kg body-wt) and total output (disintegrations/min per 4 h per kg body-wt) and specific activity (disintegrations/min per 4 h per mg protein) of radioactively labelled trichloroacetic acid (TCA)-precipitable protein secreted during 4 h stimulation with three dose combinations of cholecystokinin-pancreozymin (CCK) and secretin in rats given heat-inactivated soya-bean flour (□) and raw soya-bean flour (▨). Mean values and standard deviations, represented by vertical bars, for three rats/treatment.

three doses of CCK were reached within the first hour after the start of stimulation with CCK, the maximal rates of incorporation of L-[^{14}C]leucine were reached after about 3 h, at a time when total proteins secretion was progressively decreasing (Figs. 2 and 3).

DISCUSSION

Under the conditions of the present study, the rats given RSF differed from the control animals by secreting more stored, unlabelled protein during stimulation

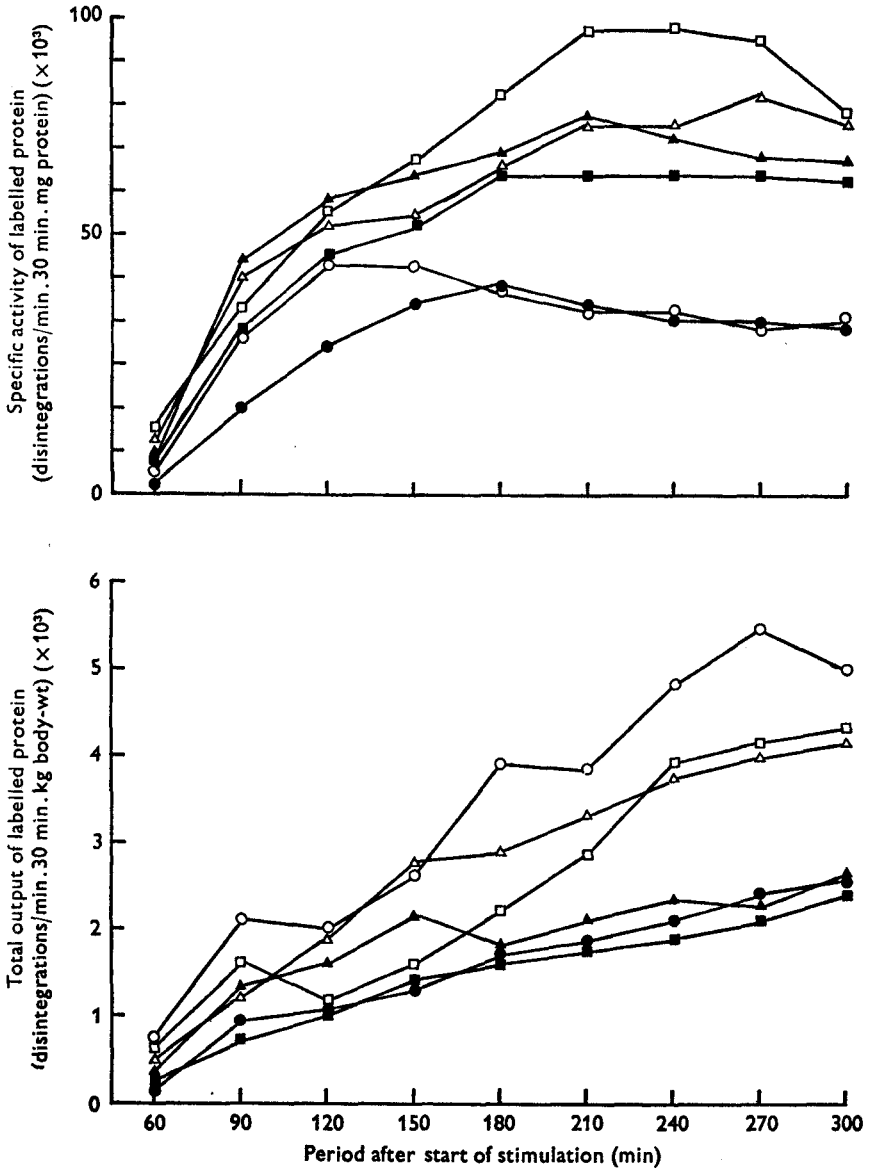

Fig. 2. Time-course of the incorporation of L-[1-^{14}C]leucine into the trichloroacetic acid-precipitable proteins secreted during 4 h stimulation with three dose combinations of cholecystokinin-pancreozymin (CCK) and secretin in rats given raw soya-bean-flour (●, ■, ▲) and heat-inactivated soya-bean flour (control) (○, □, △). ●, ○, 7·5 IU CCK+0·5 CU secretin/kg body-wt per h; ■, □, 6·0 IU CCK+0·5 CU secretin/kg body-wt per h; ▲, △, 240 IU CCK+0·5 CU secretin/kg body-wt per h. Each point represents the mean of results from three rats.

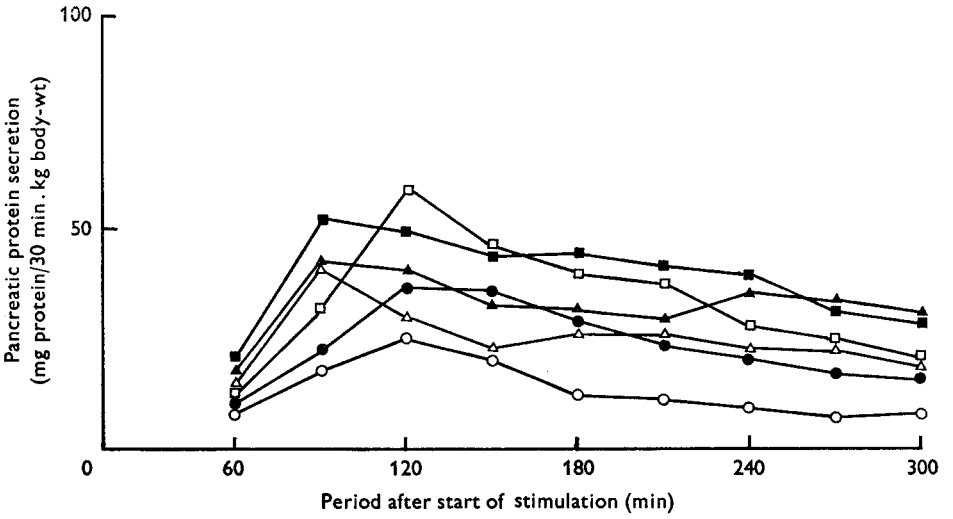

Fig. 3. Time-course of pancreatic protein secretion during 4 h stimulation with three dose combinations of cholecystokinin-pancreozymin (CCK) and secretin in rats given raw soya-bean-flour (●, ■, ▲) and heat-inactivated soya-bean flour (control) (○, □, △). ●, ○, 7·5 IU CCK+0·5 CU secretin/kg body-wt per h; ■, □, 60 IU CCK+0·5 CU secretin/kg body-wt per h; ▲, △, 240 IU CCK+0·5 CU secretin/kg body-wt per h. Each point represents the mean of results from three rats.

with sub- and supramaximal dose rates of CCK (7·5 and 240 IU/kg body-weight per h, respectively). The greater capacity to secrete pancreatic enzymes in response to CCK in rats given RSF has been noted previously (Fölsch & Wormsley, 1974). As a result of the greater secretion of unlabelled protein, the specific activity of the proteins secreted in response to these two dose rates of CCK was significantly lower in the RSF-fed rats than in the controls, although the total output of the newly synthesized, labelled proteins was not different from that of the controls.

In response to the dose rate of CCK which in the present, as in a previous study (Fölsch & Wormsley, 1973), produced maximal rates of secretion of protein (i.e. 60 IU/kg body-weight per h), both the specific activity of the secreted protein and output of the [^{14}C]leucine-labelled protein was significantly lower in the RSF-fed rats than in the control HSF-fed rats. The lower secretion of newly synthesized protein in response to the dose of CCK normally providing maximal stimulation may represent reduced sensitivity of the processes resulting in protein synthesis in the RSF-fed rats. Alternatively, it may indicate inhibition of secretion of the labelled protein by RSF during maximal hormonal stimulation. The mechanism of such inhibition has not been defined, but it seems improbable that the inhibition is attributable to intracellular feedback from the large stores of zymogen granules which accumulate during feeding of RSF (Fölsch *et al.* 1974*b*) since these stores are at their lowest during stimulation with the dose rate of CCK which produces maximal rates of pancreatic secretion (Fölsch *et al.* 1974*b*).

The results of the present study also indicated that the rate of synthesis and secretion of new proteins from the pancreas did not compensate for the decrease

in secretion of pre-formed proteins from the gland during prolonged stimulation with all dose rates of CCK. After stimulation for 90–120 min, the total secretion of protein invariably decreased with time, even though the rate of stimulation with CCK was constant, in part because secretion of new proteins assumed an almost constant rate and did not continue to increase with time. It seemed improbable that deficient pools of precursor amino acids were primarily responsible for limitation of the rate of synthesis of new proteins, since the results of the present study indicated that the rate of secretion of new proteins could be increased during the last 2 h of stimulation with CCK by increasing the dose of the CCK (from 7·5 to 60 IU/kg body-weight per h).

Whatever the mechanism of the 'fall-off' in the rate of secretion of protein during stimulation, there was a relative increase in the secretion of the newly synthesized compared with the pre-existing unlabelled protein during the phase of decreasing output of total protein, even though morphological findings indicate that the pancreatic acinar cells are full of zymogen granules at the time when enzyme secretion is decreasing (Fölsch *et al.* 1974*b*). These results are compatible with the suggestion (Schramm, Sharoni & Eimerl, 1976) that the well-ordered sequential fusion–fission processes which result in the secretion of old zymogen granules from the proximity of the luminal membrane of the acinar cell are disturbed. In consequence, there is randomization of the secretion of old and newly synthesized zymogen granules, caused by penetration of luminal membrane into the cells during prolonged, intense stimulation with CCK. Alternatively, the results of the present study are compatible with the recent proposal that during treatment with trypsin inhibitor, there is increased storage of intracellular enzymes in the pancreatic cells in non-zymogenic form (Rothman, 1970) and that secreted pancreatic enzymes may be derived from more than one intracellular pool or may be secreted by mechanisms other than extrusion of zymogen granules (Rothman, 1975; Rothman & Isenman, 1974), a pathway which has also been postulated for the normal pancreatic β-cell (Track, Frerichs & Creutzfeldt, 1974) and for the insulinoma cell (Creutzfeldt, Arnold, Creutzfeldt, Deuticke, Frerichs & Track, 1973).

The authors wish to acknowledge the expert technical assistance of Mrs F. Dear. U.R.F. gratefully acknowledges a personal grant (Fo 73/1) from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, Germany. K.G.W. is a recipient of a grant from the Scottish Hospital Endowments Research Trust and from the Smith, Kline & French Foundation.

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Printed in Great Britain