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PROPERTIES OF KALLIKREIN-CONTAINING GRANULES ISOLATED FROM THE SUBMAXILLARY GLAND OF THE GUINEA-PIG

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SUMMARY

1. Granular fractions of high purity consisting of subcellular kallikrein- and amylase-storing organelles have been isolated from homogenates of guinea-pig submaxillary gland.

2. The isolated kallikrein- and amylase-containing granules closely resembled secretory granules observed *in situ* in serous acinar cells in intra-granular appearance, size and histochemical reaction.

3. The subcellular, histochemical and ultrastructural studies indicate that the serine protease, kallikrein, is like amylase an exocrine enzyme with a functional role in saliva.

INTRODUCTION

In 1966, Bhoola & Ogle reported that kallikrein in the submaxillary gland of the guinea-pig is stored in cytoplasmic particles. Subsequently using sucrose density-gradient analyses Bhoola (1968) showed that kallikrein was sequestered in distinct organelles which differed from both lysosomes and mitochondria in their sedimentation rates.

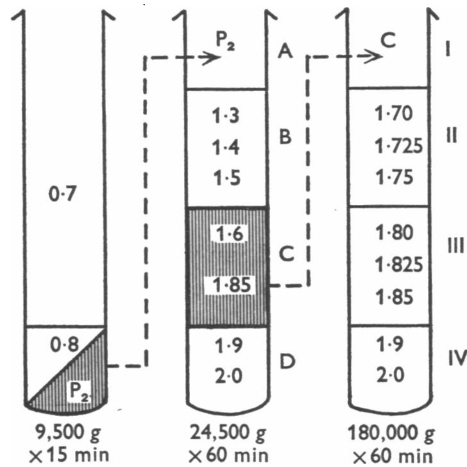
The present experiments were designed to differentiate further kallikrein-containing granules from other subcellular populations, to determine their ultrastructural and histochemical properties and to characterize chemically kallikrein sequestered within the secretory organelles. The results of this study together with the observations on the ultrastructure of the acinar secretory cell *in situ* (Heap & Bhoola, 1970) support the view that the physiological function of kallikrein is associated with an exocrine rather than an endocrine role in the submaxillary gland (see Schachter, 1969).

A preliminary account of this work has been published (Bhoola & Heap, 1970).

METHODS

Preparation of homogenates

In each experiment submaxillary glands from six guinea-pigs were removed, rinsed in 0·7 M sucrose, dried on filter paper and cut into small pieces. The glands were homogenized for 2 min with an Ultra-turrax homogenizer (generator diameter 18 mm; the circumferential speed of the generator was controlled by a Thyristor). The homogenate was centrifuged at 370 *g* for 5 min in a B.T.L. centrifuge; the precipitate was gently rehomogenized five times using the same procedure. The mean concentration of fresh glandular tissue in the homogenate was 46 mg/ml.



Text-fig. 1. Differential and density-gradient centrifugation.

Centrifugation

Separation of the nuclear fraction (P_1) was carried out on a B.T.L. centrifuge at 660 *g* for 5 min. The supernatant was layered on a 1·5 ml. cushion of 1 % glycogen and 0·8 M sucrose and centrifuged at 9500 *g* for 15 min in an M.S.E. 65 centrifuge. The precipitate containing secretory granules and mitochondria (fraction P_2) was resuspended in 0·8 M sucrose and 1·5 ml. was layered on a 4 ml. sucrose column with a gradient extending from 1·3 to 2·0 M sucrose. The junction of each sucrose layer was marked and after centrifugation at 24,000 *g* for 60 min the tubes (cellulose nitrate, Beckman) were cut with a specially designed cutter in order to recover the fractions containing the various subcellular elements. 1·0 or 1·5 ml. of the secretory granule fraction (fraction C, see Text-fig. 1) separated on the first gradient was layered on a second 4 ml. sucrose column with a gradient spread from 1·7 to 2·0 M sucrose and the tubes centrifuged at 180,000 *g* for 60 min.

Gradients were prepared 1–2 hr before use. The gravitational effect (*g*) was calculated using maximum radius values.

In each experiment aliquots of all fractions were taken for biochemical estimations. For the morphological studies a proportion of the fraction was recentrifuged to form pellets.

Electron microscopy

The pellets were fixed immediately after centrifugation. In separate experiments the fixatives used were as follows: (*a*) 1 % osmium tetroxide for 2 hr; (*b*) 2·5 % (w/v) glutaraldehyde −4 % paraformaldehyde −2 % acrolein (triple aldehyde) mixture for 2 hr followed by 1 % osmium tetroxide for 2 hr; (*c*) triple aldehyde mixture for 3 hr. Both the osmium tetroxide and the triple aldehyde mixtures contained sucrose (the molarity of the sucrose solution corresponding to that in the fraction from which the pellet was formed) and Sörensens phosphate buffer (pH 6·0; 1/15 M-KH_2PO_4-1/15 M-NaH_2PO_4). After dehydration in graded alcohols and 1,2-expoxypropane, the pellet was embedded in Araldite. The time from removal of the glands to the embedding of the pellets was continuous and usually took 18–24 hr.

Silver-gold sections cut on an LKB ultramicrotome were stained with uranyl acetate and lead citrate and examined in a Hitachi HU 11B or Siemens 1 Elminskop microscope.

Histochemistry

The pellets for histochemistry were fixed only in the aldehyde-acrolein mixture already described. Sections were cut serially for light (± 1 μm) and electron microscopy. The sections for light microscopic histochemistry were stained with the following reagents: (*a*) periodic acid-Schiff reagent, PAS (see Pearse, 1968); (*b*) toluidine blue-sodium borate solution (Richardson, Jarrett & Finke, 1960).

Measurement of enzymes and protein

Kallikrein was measured by its ability to release kallidin from a substrate prepared from heated (61° C, 3 hr), dialysed dog plasma; both kininase and prekallikrein are destroyed in plasma treated in this way (Werle, Götze & Keppler, 1937). The substrate and enzyme were added without prior incubation to an organ bath which had a constriction (1 mm internal diameter) at its base. The activity of the released kallidin was assayed on the isolated guinea-pig ileum in Mg-free Tyrode solution at 35° C and bubbled with 95 % O_2–5 % CO_2. Atropine (10^{-8} g/ml.), mepyramine (10^{-8} g/ml.) and L-cysteine (2×10^{-4} g/ml.) were added to the bath before each test.

Serine hydrolases with trypsin-like enzyme activity were measured on the synthetic substrate benzoylarginine-*p*-nitranilide (BAPA). The assay medium consisted of 0·2 ml. enzyme (homogenate, subcellular fractions or aqueous extracts of freeze dried glands), 0·1 ml. BAPA (0·01 M) and 2·7 ml. Tris-HCl-$CaCl_2$ buffer (0·2 M Tris, 0·025 M-$CaCl_2$, pH 7·8). The rate of hydrolysis was followed at 25° C at 390 nm on a Unicam SP 800 spectrophotometer using a special cell compartment designed to measure absorbance changes in turbide solutions.

Amylase was measured by the iodine titration method. Kenetic studies in which both substrate (soluble starch) and enzyme (Hog pancreatic amylase, crystalline type 1-A; Sigma) concentrations were varied indicated that the amyloclastic method used in these experiments is suitable for quantitative measurement of amylase (R. Lancaster, H. Calvert & K. D. Bhoola, unpublished). Furthermore, similar quantitative results were obtained using a saccharogenic procedure (Searcy, Hayashi & Berk, 1966). Additions of albumen and globulin in final concentrations of 10 mg/ml. to the incubation medium did not reduce amylase activity measured by the iodine titration method (see Searcy, Hayashi, Hardy & Berk, 1965).

For the amylase assay subcellular fractions were added to a medium consisting of 5·0 ml. 1 % soluble starch solution, 2·0 ml. 1 % sodium chloride and 2 ml. Sörensens

phosphate buffer (pH 6·9), and incubated at 38° C. Appropriate controls were carried out to determine the effect of glycogen on amylase activity.

Zero time was taken from the moment the enzyme fraction was added to the starch-buffer mixture. 0·1 ml. of the incubated mixture was taken every 30 or 60 sec and added to 3 ml. 0·02 N freshly made iodine solution (prepared from 0·1 N stock solution containing 30 *g* potassium iodide and 12·7 *g* iodine/l.) and read immediately on a Unicam SP 1300 Colorimeter (filter No. 6). The time taken to reach 50 % transmission was used to calculate amylase activity.

Succinate-neotetrazolium reductase was measured by the method described by Slater & Planterose (1960). The production of reduced neotetrazolium chloride (formazan) by mitochondria in the subcellular fractions was followed in the presence of disodium succinate, vitamin C and vitamin K_3. The standard succinate mixture consisted of 20 ml. 0·1 M sodium phosphate buffer (pH 7·4), 2 ml. 0·5 M disodium succinate and 1 ml. 0·1 M sodium ethylenediamine tetra-acetate; blank values were determined by substituting disodium malonate for succinate in the standard buffer mixture. The vitamin K_3 suspension was always freshly made and prepared in the following way: 12·5 mg vitamin K_3 and 0·5 ml. ethanol (Analar) were added to 12 ml. 0·2 % bovine albumin in 0·1 M sodium phosphate buffer (pH 7·4).

1 ml. enzyme fraction, 1 ml. standard buffer mixture and 0·2 ml. vitamin C (2 μ-mole) were incubated at 37° C. After 2 min 0·1 ml. vitamin K_3 suspension and 0·2 ml. neotetrazolium chloride (2 % aequeous solution) were added. The reaction was stopped after 30 min with 1 ml. ice-cold 10 % trichloroacetic acid. The formazan was extracted with 5 ml. ethyl acetate. The tubes were centrifuged and the ethyl acetate extract was measured at 510 nm on a S.P. 500 Unicam spectrophotometer.

Acid phosphatase was measured by incubating 0·5 ml. enzyme fraction, 0·5 ml. disodium *p*-nitro-phenyl orthophosphate (0·005 M in citrate buffer), 0·1 ml. 0·5 % Triton-X 100 and 1·0 ml. 0·3 M citrate buffer at 37° C for 60 min. The reaction was stopped with 1 ml. 8 % trichloroacetic acid. Colour was developed with 15 % Na_2CO_3. The tubes were centrifuged and the supernatant read at 420 nm on Unicam S.P. 500 spectrophotometer.

β-Glucuronidase activity was determined by incubating 0·5 ml. enzyme fraction, 0·5 ml. phenolphthalein-β-D-glucuronide (0·025 M in acetate buffer), 0·1 ml. 0·5 % Triton-X 100 and 1·0 ml. 0·3 M acetate buffer (pH 5·0) at 37° C. After 1 hr the reaction was terminated by adding 4 ml. alkaline reagent (pH 10·7; 1 M-Na_2CO_3, 0·2 M glycine and 0·2 M-NaCl). The mixture was centrifuged and read at 550 nm on a Unicam S.P. 500 spectrophotometer.

Protein was measured by the method of Lowry, Roseburgh, Farr & Randall (1951).

Electrophoresis

Further identification of kallikrein and amylase in the secretory granule fractions of the first and second sucrose density-gradients was carried out by paper electrophoresis. Fractions suspended in sucrose were divided into aliquots, to one of which was added Triton-X 100. The fractions and a highly purified preparation of porcine submaxillary kallikrein (1000 KE/mg protein, kindly provided by Professor E. Werle) were applied to the centre of strips of Whatman No. 1 paper 5 cm wide and 35 cm long. The electrophoresis was carried out in barbitone buffer (pH 8·6) at 4° C for 16 hr (120 V, 2·5 mA). The papers were dried at room temperature, folded and divided longitudinally into two equal sections, which were cut into 2·5 cm strips; the strips from one section were eluted with Tyrode solution for estimating kallikrein and the other with Sörensens buffer (pH 6·9) for measuring amylase.

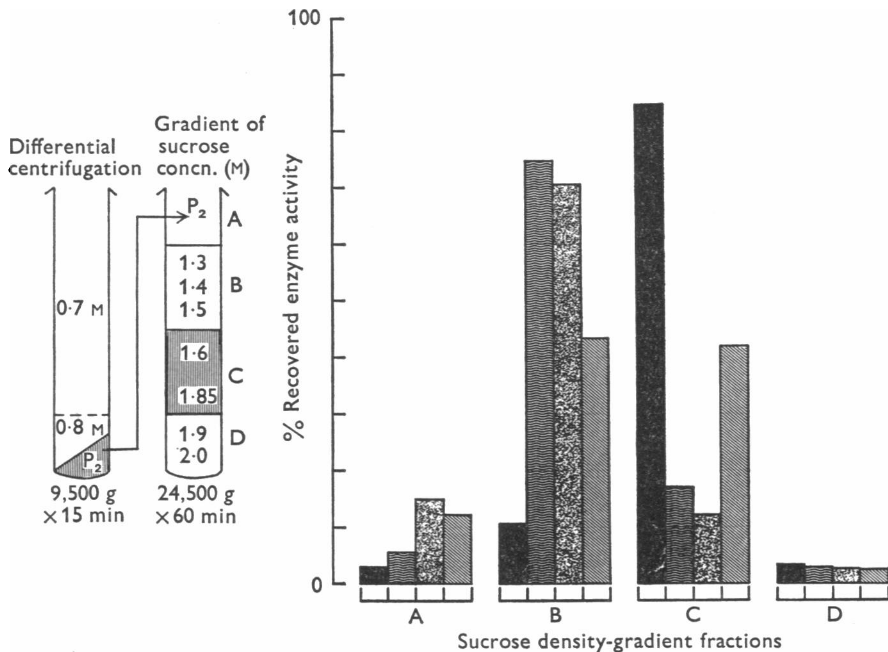
Definition of activity

The activity of each enzyme and of protein in the subcellular fractions was at first calculated as a percentage of additive (total) recovery (% *R*). In the second gradient, activity was initially determined as % *R* and then expressed as a percentage of the activity in fraction C. The recovered activity (% *R*) of kallikrein and amylase in each fraction was also expressed as a percentage of P_2 and P_2C (see Text-fig. 1). The relative specific activity (RSA) was calculated by dividing the percentage recovered activity (% R) by the protein content of the same fraction expressed as a percentage of recovered protein.

RESULTS

Isolation of kallikrein granules

Organelles containing kallikrein were initially separated on a cushion of sucrose and glycogen in order to minimize the rupture of secretory granules which occurs as a result of *stickiness* between the limiting membrane of the

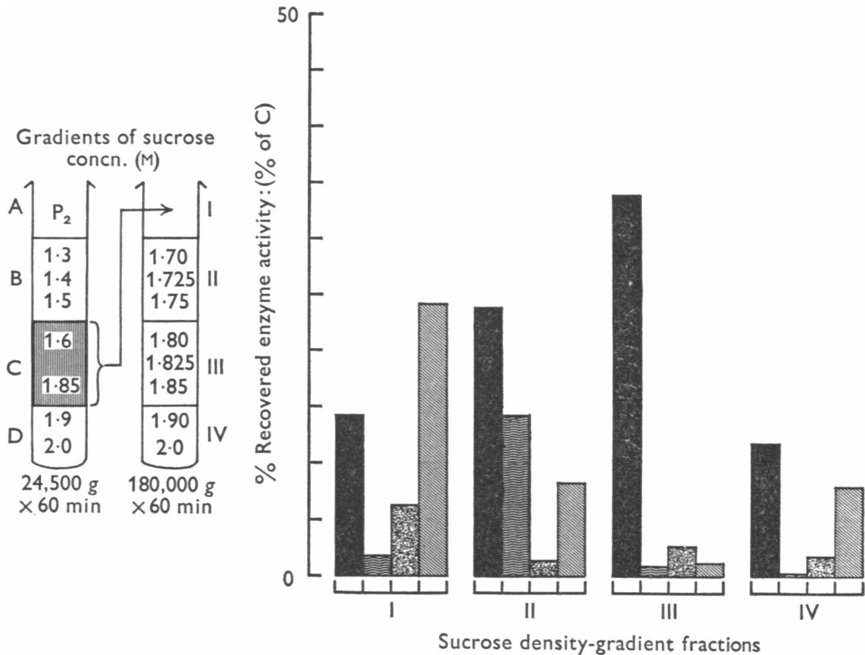


Text-fig. 2. Separation of subcellular organelles on the first sucrose density-gradient. (■) = kallikrein; (▤) = succinate-neotetrazolium reductase; (▦) = β-glucuronidase; ▧ = acid phosphatase. Mean results of three experiments.

storage granules (Maunsbach, 1966). The subcellular organelles recovered at 9550 *g* were layered on the first discontinuous sucrose density-gradient. After centrifugation the first gradient usually contained four distinctly

visible particulate zones, two in fraction B, one in fraction C and one in fraction D. The cellulose nitrate centrifuge tubes were cut into four sections and the various subcellular elements recovered in successive sub-fractions (see Text-fig. 1).

Mitochondrial succinic-neotetrazolium reductase and lyosomal β-glucuronidase and acid phosphatase were mostly localized in fraction B (1·3–1·5 M sucrose) (refer Pl. 1*A* and 1*B*), whereas kallikrein was consistently recovered from fraction C (1·6–1·85 M sucrose) (Text-fig. 2, refer



Text-fig. 3. Isolation of kallikrein-containing granules on the second sucrose density-gradient. Shading as for Text-fig. 2. Mean results of three experiments.

Pl. 2*A*). Considerable purity of the fraction containing the secretory granules was achieved by centrifuging fraction C on the second discontinuous sucrose density-gradient. This gradient which generally consisted of two visible particulate zones was also divided into four sections (refer Text-fig. 1). The kallikrein-containing secretory granules attained equilibrium mainly with 1·8–1·85 M sucrose (fraction III) and were clearly separated from the other subcellular elements (Text-fig. 3, refer Pl. 3).

Comparison of the subcellular distribution of kallikrein with that of amylase

In previous experiments using differential centrifugation, kallikrein was recovered mainly from subcellular particulate fractions whereas amylase was predominantly solubilized and less than 10% of its activity was recovered from the granule fractions (Bhoola & Ogle, 1966; Bhoola, 1968). Although in the present experiments more gentle homogenization techniques and procedures designed to obtain an increased recovery of storage granules were used, the major amylase activity was still found in the supernatant. The difference in recovery in the particulate fractions supported the view that amylase and kallikrein were sequestered in similar but separate granules. Attempts to differentiate the organelles containing these two enzymes on sucrose density-gradients were, however, unsuccessful. When the storage particles separated at 9500 *g* were centrifuged on the first sucrose density-gradient, the amylase granules showed the same distribution pattern as the kallikrein granules (Table 1). Moreover, when the secretory granules in fraction C were centrifuged on the second gradient no clearer differentiation was obtained between the organelles containing these enzymes (Table 2).

TABLE 1. Localization of kallikrein and amylase on the first sucrose density-gradient

Sucrose concn. (M)	Sub-fractions	Kallikrein			Amylase		
		%*R*	RSA	%P_2	%*R*	RSA	%P_2
P_2	A	4·2 ± 1·2	0·33	2·2	19·7 ± 6·1	1·17	5·1
1·3 1·4 1·5	B	11·1 ± 1·4	0·40	6·0	7·0 ± 2·2	0·24	1·8
1·6 1·85	C	82·9 ± 2·5	1·92	43·7	77·3 ± 7·0	1·62	20·2
1·9	D	2·1 ± 0·7	0·44	1·1	3·0 ± 1·1	0·61	0·7

%*R*: % additive recovery; RSA: relative specific activity; %P_2: % additive recovery (%*R*) expressed as percentage of fraction P_2. Mean results of four experiments ± S.E. of mean.

Morphology of the subcellular organelles separated on sucrose density-gradient

Ultrastructure. The particulate zone in fraction B in equilibrium with 1·3 M sucrose consisted of microsomal membranes and a few storage granules (Pl. 1*A*). The region of fraction B associated with 1·4 M sucrose contained predominantly mitochondria (Pl. 1*B*). In this fraction particles

resembling the secretory organelles in size and electron-density, observed *in situ* in the intercalated duct cells (Heap & Bhoola, 1970), appeared to be present in greater numbers than acinar secretory granules.

Fraction C which in the biochemical studies had the highest kallikrein and amylase activities (refer Table 1) consisted mainly of storage granules (Pl. 2*A*). Although the electron-pale and -dense granules observed *in situ* in acinar cells (Pl. 2*B*) were both identified in fraction C, the dense granules appeared to form the major population. The membrane of the few electron-pale granules in this fraction was frequently incomplete or absent. Furthermore, the pale particles were particularly prone to rupture and distintegrate.

TABLE 2. Distribution of kallikrein and amylase on the second sucrose density-gradient

Sucrose concn. (M)	Sub-fractions	Kallikrein %R	Kallikrein %C	Kallikrein %P_2C	Amylase %R	Amylase %C	Amylase %P_2C
C	I	16·9 ± 2·1	14·2	7·5	9·1 ± 3·5	7·0	1·8
1·70 1·725 1·75	II	28·3 ± 3·9	23·4	12·3	25·6 ± 9·4	17·9	4·6
1·80 1·825 1·85	III	41·7 ± 1·3	34·5	18·2	51·2 ± 10·0	39·6	10·3
1·9 2·0	IV	12·6 ± 5·9	10·4	5·5	14·0 ± 4·0	10·0	2·8

%R: % additive recovery; %C: % additive recovery (%R) expressed as percentage of fraction C; %P_2C: % additive recovery (%R) expressed as percentage of P_2C. Mean results of four experiments ± S.E. of mean.

A granular fraction of satisfactory purity was obtained in the second gradient. Mitochondria and microsomal membranes which were regularly present as contaminants in the secretory granule fraction C were separated from these organelles and formed a distinct particulate zone at 1·7 M sucrose. Most of the kallikrein- and amylase-containing organelles, mainly freed of contaminating subcellular elements, were in fraction III in equilibrium with 1·8–1·85 M sucrose (refer Table 2). Furthermore the size, intragranular structure and histochemical reactivity of the isolated enzyme-containing organelles were similar to that of the secretory granules observed *in situ* in the luminal region of the serous acinar cells (see Heap & Bhoola, 1969; Heap & Bhoola, 1970).

Histochemistry. Pellets prepared from the purest secretory granule fractions were fixed in triple-aldehyde mixtures (see Methods) and processed for electron microscopy; serial sections of alternate thickness were

examined by electron microscopy ($\pm 0{\cdot}05$ μm) and light microscopic histochemistry (± 1 μm). Populations of the isolated organelles similar to those visualized by electron microscopy and illustrated in Pl. 3 reacted positively with PAS and toluidine blue.

Chemical studies on kallikrein in the isolated secretory organelles

Hydrolysis of benzoyl arginine-p-nitranilide (BAPA). Several serine proteases (three trypsin-like and four kallikrein isoenzymes) have been identified in extracts of the submaxillary gland of the rat (Riekkinen, Ekfors & Hopsu, 1966). One of the trypsin-like enzymes (salivain) resembled kallikrein in its ability to produce hypotension and to increase capillary permeability in rabbits (Ekfors, Hopsu-Havu & Malmiharju, 1969). The serine proteases associated with kallikrein are probably also sequestered in secretory organelles. It was therefore important to examine whether trypsin-like enzymes occur in the submaxillary gland of the guinea-pig. The purest secretory granule fraction with the highest kallikrein activity and aequeous extracts of freeze-dried guinea-pig submaxillary gland (0·5 ml., 500 mg/ml.) were completely ineffective in hydrolysing BAPA.

Comparison of the electrophoretic mobility of kallikrein and amylase. Although the early experiments clearly indicated that kallikrein was stored intracellularly in granules, the question whether kallikrein is sequestered as a *free* or *bound* enzyme within the granule had not been examined (see Bhoola & Ogle, 1966).

Kallikrein in the isolated granules obtained from fraction III was solubilized by adding Triton-X 100. Aliquots of the enzyme solution with and without Triton-X 100 were applied to Whatman No. 1 paper and the electrophoretic mobility of kallikrein and amylase determined. In solutions treated with Triton-X 100 kallikrein migrated as a wide zone towards the anode with maximum enzyme activity 8·0–9·0 cm from the isopotential mark whereas amylase moved as a single band in the opposite direction 9·0–10·0 cm towards the cathode. Without Triton-X 100 both enzymes showed very little migration from the isopotential point although the direction of movement was identical to that already described. Furthermore, the magnitude and direction of the electrophoretic movement of kallikrein solubilized from the isolated granules was similar to that obtained with purified preparation of porcine submaxillary kallikrein (1000 KE/mg protein) (see Moriya, Pierce & Webster, 1963).

DISCUSSION

The present study has clearly yielded very pure granule fractions which also contained the highest kallikrein activity. Purity was assessed by electron microscopy and by measurement of enzymes specific to sub-cellular organelles. The separation of the contaminating intracellular membranes and particles was mainly achieved by refloating the secretory organelles recovered from the first gradient on to a second gradient with a narrower range of sucrose concentration.

In previous experiments isolated organelles containing kallikrein resembled pancreatic secretory (zymogen) granules in their sedimentation rate and histochemical reaction (Bhoola, 1968). This finding indicated that the organelles containing kallikrein were derived from the acinar cells of the submaxillary gland. The cellular origin of the kallikrein granules was therefore investigated in the intact gland by both light and electron microscopy. The histochemical reactivity of the isolated kallikrein particles with PAS and toluidine blue was similar to that observed with secretory organelles in the acinar and intercalated duct cells in the intact submaxillary gland (Heap & Bhoola, 1969, 1970). In view of this finding the question arose as to which cell type contained the kallikrein granules. The storage organelles (mean diameter *in situ*, 0·2–0·6 μm) found in the intercalated duct cells were mainly associated with mitochondria (mean diameter *in situ*, 0·7–1·1 μm). In contrast, the secretory granules from the serous acinar cells identified by their size (mean diameter *in situ*, 1·0–2·6 μm), ultrastructural appearance and histochemical reactivity, sedimented predominantly in the fractions which contained the highest kallikrein activity. The kallikrein-containing organelles therefore originated almost certainly from the serous acinar cells.

Since in differential centrifugation experiments amylase was mainly solubilized, it was assumed that amylase was stored intracellularly in labile organelles which ruptured readily during homogenization and centrifugation of the gland tissue. Kallikrein, however, seems to be sequestered in more stable granules since in the same experiments it was recovered mainly in particulate fractions. The possibility that the two enzymes were sequestered in separate organelles was carefully tested but the centrifugation experiments failed to differentiate between organelles containing kallikrein and amylase.

In the very first study on the subcellular localization of kallikrein in the submaxillary gland its *biological* activity, indicated by its ability to release kallidin from its natural plasma substrate (γ_2 globulin), was determined by assays on the guinea-pig ileum, rat duodenum and dog blood pressure (Bhoola & Ogle, 1966). Because trypsin-like serine proteases with biological

activity similar to that of kallikrein have been recently identified in the submaxillary gland of the rat (Ekfors *et al.* 1969), kallikrein from the submaxillary gland of the guinea-pig was further characterized. However, no trypsin-like serine hydrolases were detected in the submaxillary gland of the guinea-pig.

The subcellular studies were originally designed by Bhoola & Ogle (1966) to ascertain whether knowledge of the intracellular localization of kallikrein would provide insights into the physiological function of salivary kallikrein. In the present experiments kallikrein and amylase activity was demonstrated in secretory granules. Ultrastructure studies of the submaxillary gland of the guinea-pig (Heap & Bhoola, 1970) have shown that the secretory granules containing these enzymes are mainly stored in the apical region of the serous cell, as if orientated for secretion into the acinar cell lumen; moreover, these granules have been seen to empty into the lumen. The clear association between the intracellular storage of the serine protease kallikrein and the digestive enzyme amylase in granule populations which have been seen to fuse with the luminal membrane and discharge into the acinar lumen (Heap & Bhoola, 1970) supports the view that kallikrein is an exocrine enzyme (see Werle & Roden, 1936, 1939; Feldberg & Guimarais, 1935) and therefore may have a primary functional role in saliva.

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EXPLANATION OF PLATES

PLATE 1

A. Microsomal membranes in equilibrium with 1·3 M sucrose. MV = microsomal vesicles; M = mitochondria; AG = acinar secretory granule. Pellet was fixed in 1 % osmium tetroxide for 2 hr. Section is a representative field.

B. Mitochondria (M) recovered at 1·4 M sucrose. IDG = Intercalated duct granules. Pellet was fixed in 1 % osmium tetroxide for 2 hr. Section is a representative field.

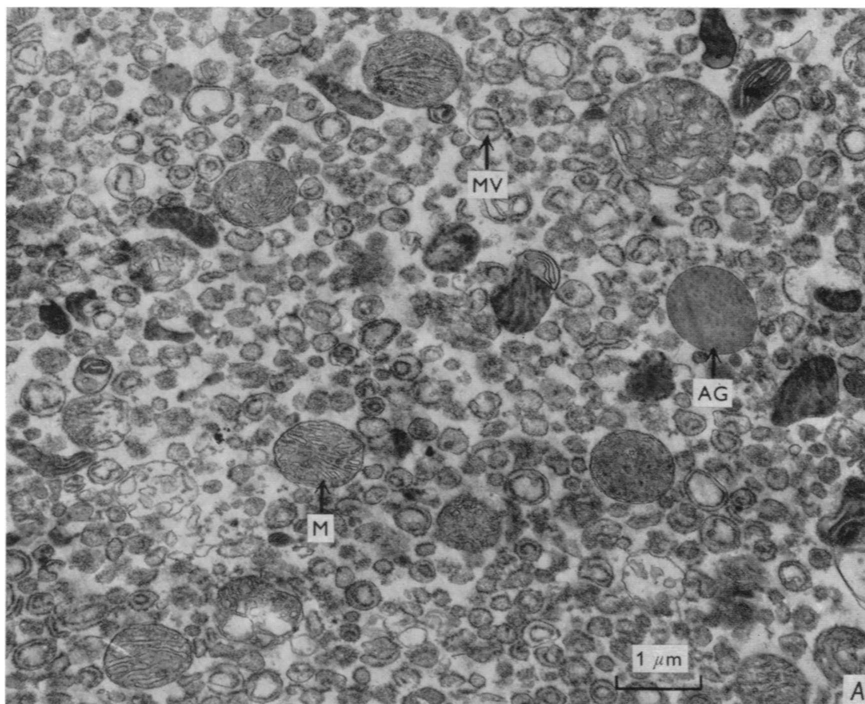
PLATE 2

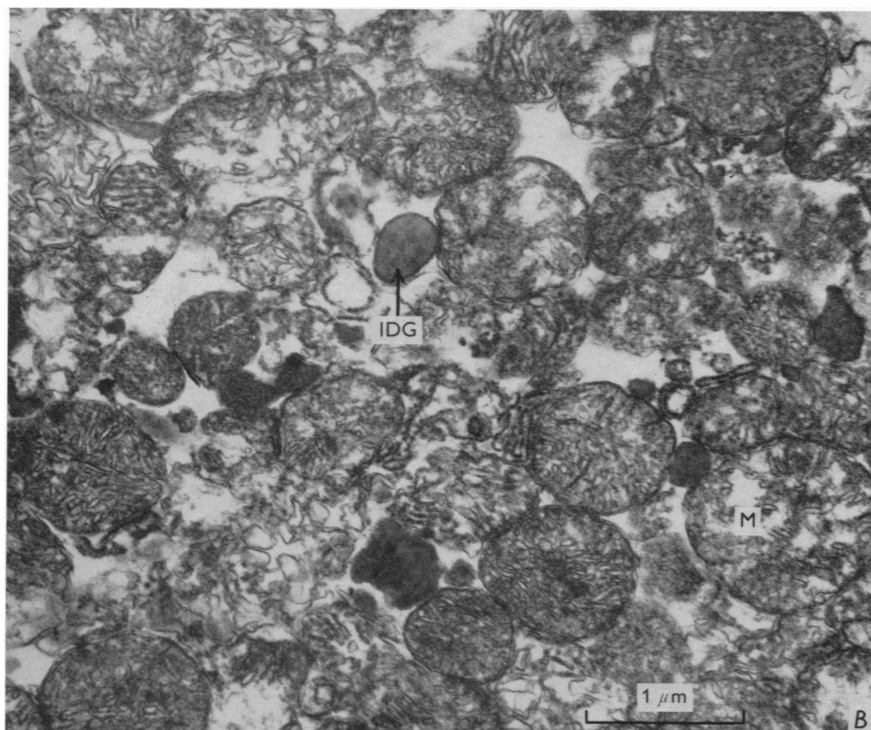
A. Serous acinar secretory granules. PG = electron-pale granule; DG = electron-dense granules. Arrows indicate secretory material from ruptured granules. Pellet fixed in triple aldehyde mixture for 2 hr followed by 1 % osmium tetroxide for 2 hr. Section is a selected field.

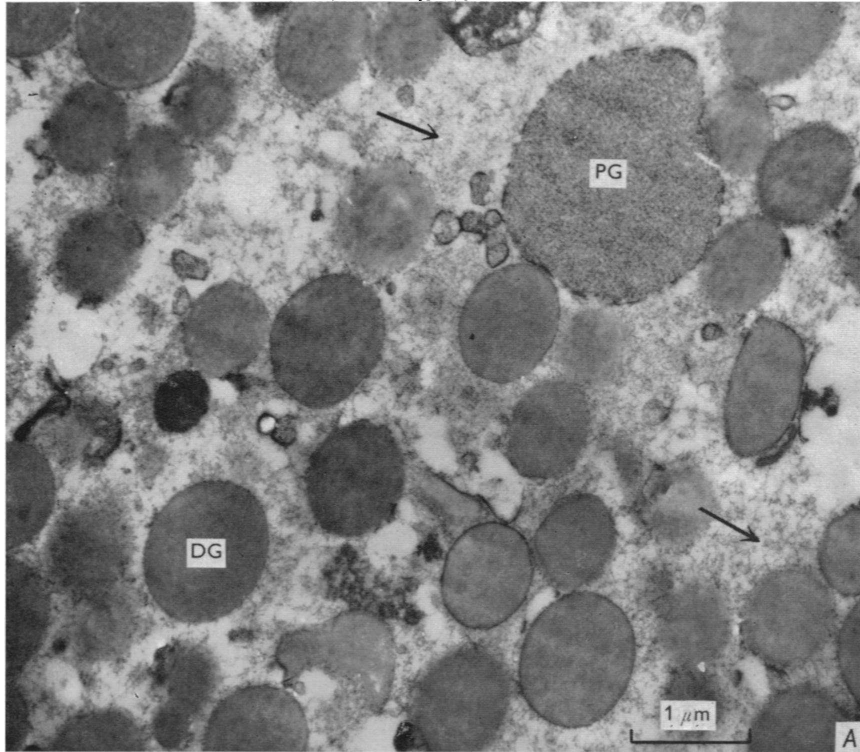
B. Apical part of serous acinar cells of the submaxillary gland of the guinea-pig showing electron-pale (PG) and electron-dense (DG) granules confined to region adjacent to the cell lumen. LU = lumen. Secretory material present in the lumen. Tissue fixed in triple aldehyde for 2 hr followed by 1 % osmium tetroxide for 2 hr.

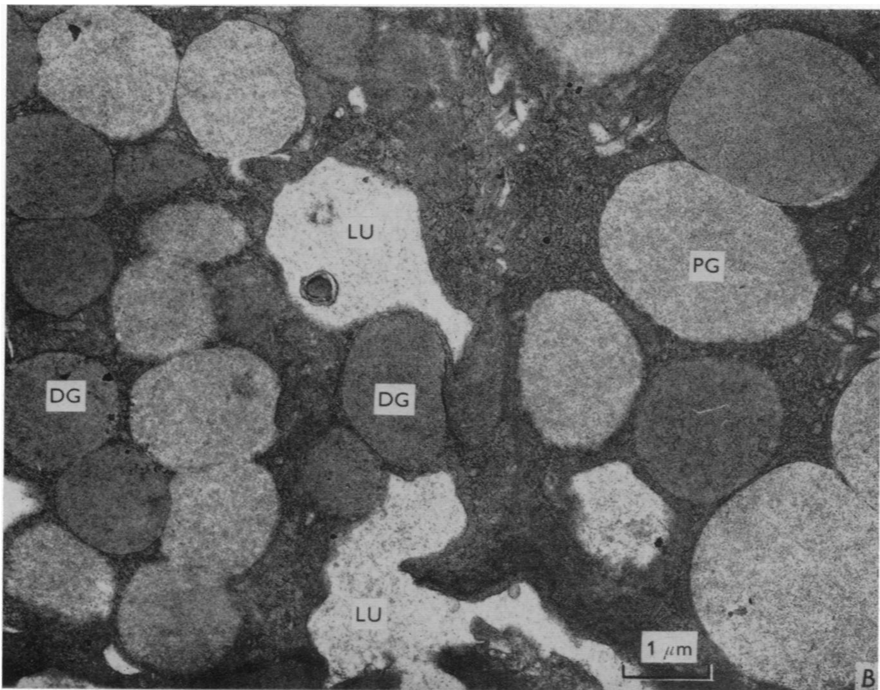
PLATE 3

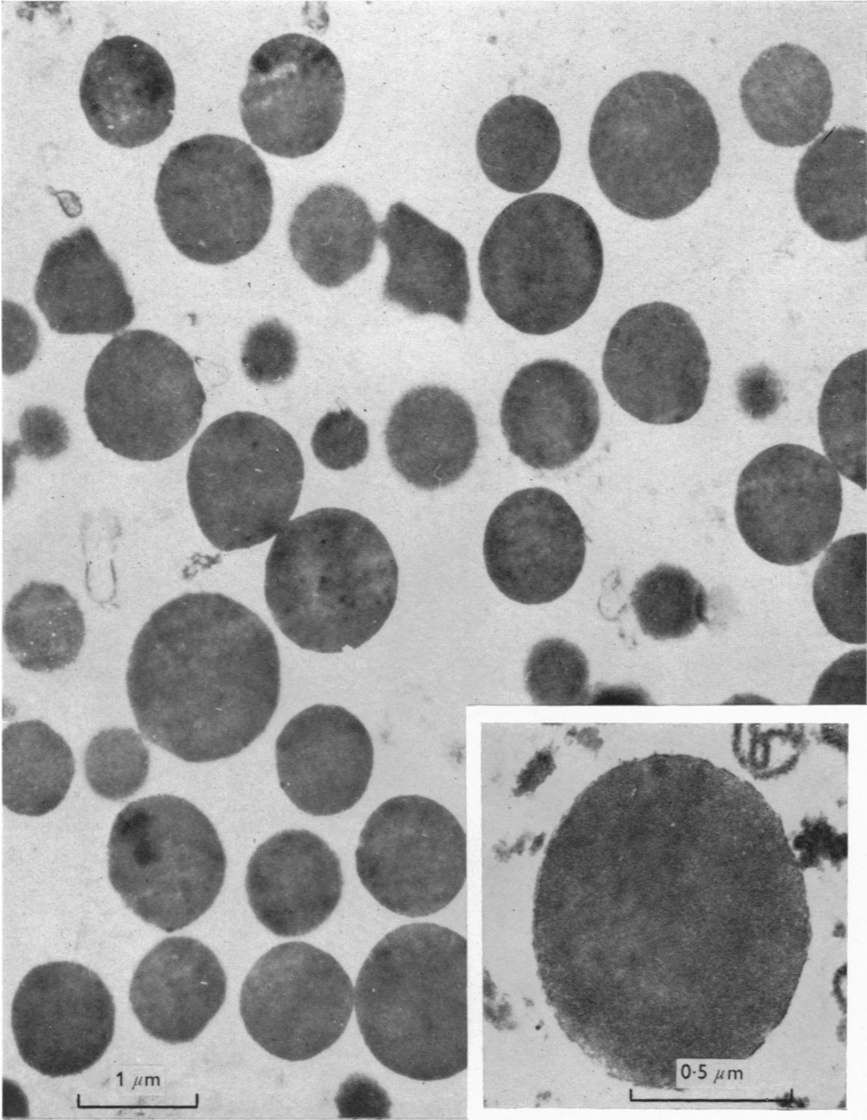
Serous acinar secretory granules in equilibrium with 1·8–1·85 M sucrose (Fraction III) which contained the highest kallikrein and amylase activity. They resemble the acinar cell granules *in situ* in size and electron density. Inset shows intragranular appearance of an isolated granule which seems to be identical with that observed in the serous acinar secretory granules in Pl. 2*B*. Pellet was fixed in triple aldehyde for 2 hr followed by 1 % osmium tetroxide 2 hr. Section is a representative field.


MV
AG
M
1 μm
A


IDG
M
1 μm
B


PG
DG
1 μm
A


LU
PG
DG
DG
LU
1 μm
B



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