EFFECT OF TRYPSIN ON LIVER MICROSOMES

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ABSTRACT

The effect of trypsin on the morphology of the rat liver microsomal fraction isolated by differential centrifugation has been investigated. The microsomes were incubated at 37°C and centrifuged thereafter under the conditions of their initial isolation. The trypsin-treated microsomes and the untreated controls were fixed in unbuffered osmium tetroxide and embedded first in gelose and then in methacrylate. In the trypsin-treated microsomes, there was a removal of the ribosomes from the rough vesicles. Parallel chemical determinations showed that the total nitrogen and total phosphorus of the pellet were lowered. Particles, densely stained with phosphotungstic acid (PTA) and homogeneous in appearance, were found within microsome smooth vesicles in a fluffy layer which collects on the top of the microsome pellet. The morphology of these PTA-stained particles remained unchanged after incubation with trypsin.

Since the work of Claude (6) on the systematic fractionation of mammalian liver cells by differential centrifugation, much attention has been devoted in many laboratories to the study of the different fractions isolated by his method. In his early work, Claude demonstrated that most of the ribonucleic acid of the cytoplasm, up to 85 per cent, was associated with the microsome fraction, and he suggested that the microsome material was responsible for the basophilia of the ground substance of the cell (7), a view that has been confirmed by later work (1, 21, 18, 12, 22).

A further step was made in our understanding of the origin and constitution of the microsome fraction by the use of the combined methods of differential centrifugation and electron microscopy, when it was shown, first, that the microsome elements represented portions of the endoplasmic reticulum (ergastoplasm), and, second, that the ribonucleic acid found in the microsome pellet was mostly concentrated in granules of small size, about 140 Å in diameter, as described by Palade (17); these ribosome particles are found charac-

teristically attached to membranes of the endoplasmic reticulum, and to the isolated microsome vesicles (25, 3, 19).

The elementary chemical composition of the microsomes was first established by Claude (6). He demonstrated that these cytoplasmic constituents were complex structures consisting mostly of phospholipids, which account for as much as 40 per cent of their mass, and of ribonucleoproteins. Precise localization of the ribose nucleic acid in the microsomes has been investigated by Palade and Siekevitz (20), who have shown that it accompanies the attached "dense granules" when these are released following disintegration of the microsome vesicles by means of deoxycholate. These observations were supported by the results of other tests in which microsomes were subjected to ribonuclease digestion. In this case the ribonucleic acid-containing particles were dissolved, whereas the membranous constituent of microsomes was preserved, and the naked vesicles could be recovered by sedimentation (20).

The object of the present paper has been to

investigate the effect of trypsin digestion on the morphology and chemical constitution of microsomes isolated by differential centrifugation. Microsomes from rat liver were chosen for the present work since this particular material has been widely investigated and its characteristics are best known. The possible effects of trypsin action on liver microsomes have been determined by two means: first, morphologically by examination of the sedimentable fraction in the electron microscope before and after trypsin treatment, and, second, chemically by determination of the nitrogen and phosphorus content of the pellets following trypsin digestion. The results indicate that trypsin digestion is followed by a partial or a complete removal of the attached dense granules (ribosomes) from the surface of the microsome vesicles, and that this effect is accompanied by a decrease in the nitrogen and phosphorus content.

MATERIAL AND METHODS

Material

Male or female albino rats of the Wistar strain, about six months old, obtained from our own animal colony, were used in the present work. Prior to removal of the liver, the animals were subjected to an 18 hour fasting, killed by a blow on the head, and bled by section of the vessels of the neck. The excised livers were placed on ice before homogenization and fractionation.

The trypsin used for digestion was obtained from the Novo Industri Laboratories, Copenhagen, Denmark. This sterilized, dialyzed, and lyophilized product, as commercially provided, contained CaCl_2 as stabilizer in the proportions of 3 mg per 97 mg of crystalline enzyme. Before use, the stabilizer was removed by appropriate dialysis of a 0.1 per cent solution of the commercial trypsin in distilled water, against distilled water, at 2°C.

Methods

In the present work, care was taken to avoid contamination of the microsome fraction by debris from the large granules or mitochondrial fraction. For this reason, the technique selected for cell fractionation was that advocated by Claude and discussed in detail by him in his original paper (6); for the same reason, in a number of experiments, the isotonic, buffered saline was replaced by a sucrose solution, a medium devised to better preserve the integrity of mitochondria (13, 24).

PREPARATION OF THE MICROsome FRACTION: The preparation of the tissue suspension was carried out according to the technique of Claude (6).

The freshly removed, chilled rat livers were passed through a masher fitted with a 1 mm mesh screen. The resulting pulp was ground in a mortar for a total time of 5 to 10 minutes.

Depending on the experiments, two types of solutions were used for extraction: (a) an isotonic, pH 7.4 buffered solution, containing 0.85 per cent NaCl and a veronal-acetate-HCl mixture to final concentration of 0.1 M; and (b) a 0.25 M, unbuffered sucrose solution. The fluid used was added while grinding to give a 10 per cent suspension, *i.e.*, 10 ml of medium per gram of fresh liver pulp.

CENTRIFUGATIONS: The procedure of fractionation was stepwise, *i.e.*, the tissue debris, the large granule fraction, and the microsome fraction were removed in succession and separately, in order to obtain, as far as possible, a clear-cut separation, especially between the mitochondria and the microsomes. The homogenates in isotonic, buffered saline were subjected to differential centrifugation according to the scheme given by Claude (6).

The fractionation of the homogenates in 0.25 M sucrose was accomplished in two main steps: (a) a centrifugation at 4900 *g* for 15 minutes in the Spinco model L centrifuge, followed by a centrifugation of the supernatant at 35,000 *g* for 5 minutes. The deposits from these two successive runs, which removed the tissue debris, the mitochondria, and part of the large microsomes, were discarded; (b) the microsome fraction was separated by a centrifugation at 35,000 *g* for 30 minutes. The resulting pellet was washed twice by successive resuspension in the same medium and centrifugation at 35,000 *g* for 30 minutes. A stock suspension of the pellet was made in a volume one-fifth that of the original supernatant, giving a 5-fold concentration of the microsome fraction, with respect to that of the original extract.

The entire procedure of fractionation was conducted in a cold room at 2–4°C, with solutions and instruments cooled at approximately the latter temperature.

INCUBATION OF THE MICROsomes WITH TRYPSIN: The quantity of microsomes in the final suspensions that were incubated was equivalent to that in the original liver homogenate. The final concentration of dialyzed enzyme in the suspension ranged from 40 mg per cent to 0.15 mg per cent. These concentrations expressed in dry weight of enzyme per dry weight of microsomes represent, respectively, 14 per cent and 0.05 per cent.

After incubation, the specimens exposed to trypsin action, and their controls, were subjected to a single centrifugation run at 35,000 *g* for 30 minutes. Dry weight and chemical determinations were conducted on both the supernatants and the sediments, and the same or similar subfractions were examined in the electron microscope.

DRY WEIGHT AND CHEMICAL DETERMI-

NATIONS: The dry weights were determined on aliquot volumes of the specimens evaporated at 80°C, dried at 110°C for 1 hour, and brought to room temperature in a desiccator, over CaCl_2 .

The total nitrogen content of the supernatant or of the resuspended pellets was determined after mineralization in presence of selenium as catalyst, and the micro Kjeldahl was carried out in a Markham apparatus (14). The total phosphorus content was determined after sulfuric acid mineralization, following Berenblum and Chain (2).

TECHNIQUES FOR ELECTRON MICROSCOPY: Different procedures for fixation of the specimens were tested in preliminary experiments. It was found that packing of the microsome material as obtained in the centrifugation pellets was not favorable for a study of the morphology of individual microsome vesicles. In order to obviate this difficulty a technique of double inclusion was applied, which includes the following steps: (a) suspension of the pellet material to be examined in a small volume of 0.25 M sucrose; (b) fixation of the microsomes in suspension by addition of equal volume of a 2 per cent osmium tetroxide solution in distilled water to provide a final OsO_4 concentration of 1 per cent, the mixture remaining in contact for 30 minutes; (c) even dispersion of the fixed microsomes in an equal volume of 2 per cent gelose solution at 40°C so that the final concentration of the microsomes in the gelose is about the same as that in the cytoplasm (except for losses due to the procedure and not taken into consideration); (d) embedding in gelose by rapid cooling of this microsome preparation to melting ice temperature, and cutting of the gelose solidified mass into small blocks about 1 mm³ in size; and (e) complementary fixation of the gelose blocks by immersion in 1 per cent osmium tetroxide in distilled water. Details of this technique have been given in a preceding paper (8).

Further treatment of the gelose-embedded microsomes included rapid washing in distilled water, dehydration by passage through 70, 94, and 100 per cent ethanol, and final embedding in a mixture of 95 per cent butyl and 5 per cent methyl methacrylate.

Staining of gelose-microsome blocks was carried out, at the absolute ethanol stage of dehydration, by immersion of the blocks for 3 hours in a 1 per cent solution of phosphotungstic acid in this solvent. Staining of the sections was performed by floating the grids supporting the sections on a lead hydroxide solution prepared according to Watson's technique (27).

The sections were cut with a Porter-Blum microtome and examined in a Siemens Elmiskop I electron microscope.

RESULTS

The morphological observations and the chemical studies have been carried out in parallel experi-

ments and under identical conditions; the results of chemical analysis, which are quite consistent, are supported by a large set of experimental data, not included in the present report.

Morphological Observations

Preliminary observations have shown that the double embedding method, *i.e.*, dispersion and embedding in gelose prior to embedding in methacrylate, produces no detectable alterations in the morphology of the microsomes, as compared with the method of fixation and methacrylate embedding of specimens obtained directly from different levels of the packed microsome pellets. Actually, the technique appears to offer two definite advantages: first, dispersion of the microsome elements gives a better opportunity to observe the morphology of the individual microsome vesicles; and, second, homogenization of the entire microsome pellet and its redispersion in gelose gives specimens more representative of the composition of the whole pellets than are specimens obtained at different levels of the packed sediment.

The following observations deal with (1) unwashed microsomes from the concentrations run; (2) washed microsomes incubated at 37°C, without trypsin (controls), referred to as "incubated microsomes"; and (3) microsomes incubated at 37°C, with trypsin added.

1. UNWASHED MICROSOMES

This fraction refers to the jelly-like red pellet obtained by extraction either with 0.85 per cent NaCl or with 0.25 M sucrose solutions. (a) In the case of NaCl extraction the microsome pellet is slightly larger in volume and paler in color than when sucrose has been used as a medium; (b) in the case of NaCl preparations, an ill defined fluffy layer is found on the surface of the microsome pellet. This layer, which slides off readily from the pellet's surface, has been examined separately.

Figs. 1 and 2 illustrate constituents of the microsome pellet proper and of the fluffy layer, respectively. The remaining illustrations (Figs. 3 to 10) are concerned exclusively with microsomes prepared in the sucrose medium.

A section of the gelose-embedded microsomes prepared in 0.85 per cent, pH 7.4 buffered NaCl solution is illustrated in Fig. 1. The main constituent is a type of swollen vesicle that is oval or round after hypotonic fixation. The various elements seen in the electron microscope may be

classified as follows: (a) rough vesicles (*rv*), the outer surface of the limiting membrane of which is dotted with small dense granules 10 to 15 μ in diameter, the so-called Palade granules or ribosomes; inside the vesicle, small granules and irregular filaments may be detected after PTA staining; (b) smooth vesicles (*sv*), generally of smaller diameter than the rough profiles; (c) agglomerates of ribosomes (*dg*); and (d) small vesicles containing a single homogeneous granule 50 μ in diameter and frequently encountered in the form of a cluster in the fluffy layer. The latter homogeneous granules are well demonstrated only after phosphotungstic impregnation and therefore will be called the PTA-stained granules (*ptg*). Another component, not illustrated in this

figure, may be found in the microsome pellet, namely, the dense bodies, tentatively identified with lysosomes (11).

Fig. 2 illustrates the particles found in the fluffy layer on the surface of the microsome pellet in the case of isotonic NaCl extraction of liver tissue. Here the elements are the same as those encountered in the microsome pellet illustrated in Fig. 1, but the smooth vesicles (*sv*) and the homogeneous PTA-stained granules (*ptg*) appear to be comparatively more frequent than the rough profiles (*rv*). The PTA-stained granules are found in smooth vesicles which appear free of Palade's granules.

Microsomes isolated from the sucrose-extracted liver are shown in Figs. 3 and 4. The shapes and

FIGURES 1 TO 4

Electron micrographs of unwashed microsomes. Fixation in 1 per cent osmium tetroxide in distilled water. Gelose embedding, followed by methacrylate embedding.

FIGURE 1

Resuspended microsome pellet, prepared in physiological saline buffered at pH 7.4; staining, phosphotungstic acid. The particles contained in the microsome pellet, prepared under the present technical conditions, appear as swollen vesicles of two types: (a) rough surfaced vesicles (*rv*) which are limited by a membrane the outer surface of which is dotted with dense granules of regular size, the ribosomes; (b) smooth surfaced vesicles (*sv*) smaller than the rough type. Dense granules of the same size as the ribosomes form agglomerates (*dg*). Homogeneous phosphotungstic acid-stained granules (*ptg*) 50 μ in diameter are present inside some vesicles. $\times 49,000$.

FIGURE 2

Resuspended fluffy layer obtained on the top of the microsome pellet, prepared in buffered physiological saline; staining, phosphotungstic acid. Rough (*rv*) and smooth (*sv*) vesicles are present in this layer, as well as a few agglomerated dense granules (*dg*). The homogeneous granules stained with phosphotungstic acid are abundant and may form clusters (*ptg*) inside smooth vesicles. Fibrillar material (*f*) of unknown nature and composed of fine fibrils and small granules is mixed with the main vesicular elements. *g*, intravesicular precipitate. $\times 49,000$.

FIGURE 3

Resuspended pellet of microsomes prepared in sucrose solution; staining, phosphotungstic acid. The two types of vesicles are present, the rough (*rv*) and smooth (*sv*). Homogeneous phosphotungstic acid-stained granules (*ptg*) are enclosed in smooth vesicles. $\times 45,000$.

FIGURE 4

Resuspended pellet of microsomes prepared in sucrose solution; staining on section, lead hydroxide. $\times 125,000$.

The dense granules are distinctly observed on the surface of the vesicles. The phosphotungstic acid-stained granules (*ptg*) do not show any internal structure. *rv*, rough surfaced vesicles. $\times 125,000$.

TABLE I

Effects of Various Treatments on Microsomes

Gross changes observed in the pellets of the trypsin-treated microsomes compared with the pellets of the washed, untreated microsomes.

Treatment of microsomes	Changes from washed untreated microsomes		
	Volume of pellet	Color of pellet	Affinity for OsO_4
a) Washed twice b) Incubated 37°C, 30 min., no trypsin	Unchanged	Unchanged	Increased
a) Washed twice b) Incubated 37°C, 30 min., with trypsin 40 mg per cent	Diminished	Much paler	Increased
a) Washed twice b) Incubated 37°C, 30 min., with trypsin 2.4 mg per cent	Diminished	Paler	Increased
a) Washed twice b) Incubated 37°C, 30 min., with trypsin 0.1 mg per cent	Unchanged	Unchanged	Increased

characteristics of the constituents are similar to those described in the microsome pellets obtained after saline extraction of the liver tissue. The staining with lead hydroxide (Fig. 4) increases the contrast, but does not show additional structural details.

2. INCUBATED MICROSOMES

In the series of experiments in which the trypsin treatment of the microsomes was studied, different controls were used. The effects of washing, and of incubating at 37°C without trypsin, were tested. Figs. 5 to 7 demonstrate that no appreciable

FIGURES 5 TO 7

Electron micrographs of washed microsomes incubated (Figs. 6 and 7) for 30 minutes at 37°C. No trypsin present. Fixation, 1 per cent osmium tetroxide in distilled water. Gelose embedding, followed by methacrylate embedding.

FIGURE 5

Resuspended microsome pellet; staining, phosphotungstic acid. This low magnification shows the homogeneous distribution of the particles obtained by gelose embedding. $\times 13,000$.

FIGURE 6

Resuspended microsome pellet; staining, phosphotungstic acid. The vesicles appear irregular after washing and incubation, but the dense granules are preserved on the surface of the rough type of vesicles (*rv*). $\times 45,000$.

FIGURE 7

Resuspended microsome pellet; staining on sections, lead hydroxide. Rough (*rv*) and smooth (*sv*) profiles are present. The homogeneous intravesicular granules (*ptg*) are not dissolved after washing and incubation. $\times 49,000$.

changes occur as a result of incubation of the microsome suspension at 37°C for periods up to 30 minutes: the Palade granules remain associated with the vesicles and the PTA-stained granules (*ptg*) exhibit their usual, characteristic density.

3. MICROSOMES INCUBATED WITH TRYPSIN

The series of controls just described were necessary to eliminate the possibility of an autodigestion of the microsomes by their own enzymatic content. The action of crystalline trypsin on the microsomes suspended in 0.25 M sucrose buffered at pH 7.4 was tested for final concentrations of the enzyme in the suspension ranging from 40 mg per cent to 0.15 mg per cent. Three concentrations will be selected for illustration: 40 mg, 2.4 mg, and 0.15 mg per cent, respectively.

The observed changes in the gross appearance of the pellets after treatment of the microsomes with trypsin have been summarized in Table I. It appeared on gross examination that the volume of the pellets changed significantly only after tryptic digestion, and for final enzyme concentrations higher than 0.15 mg per cent. The color, which paled after simple washings, became still paler after the active tryptic digestion. Comparison of blocks, about the same in size, of gelose-embedded microsomes under a binocular micro-

scope revealed clearly that the affinity of the microsomes for osmium tetroxide is increased by simple incubation at 37°C for 30 minutes without trypsin. The same increased affinity for osmium tetroxide is also observed in the case of trypsin-treated microsomes in gelose blocks. Examined under the electron microscope, the different degrees of alteration ascribed to the effect of trypsin are illustrated in Figs. 8 to 10. The electron micrographs of Figs. 8 and 9 show gelose-embedded microsomes which were treated with 40 mg and 2.4 mg per cent trypsin, respectively. The striking effect of these concentrations of enzyme is the disappearance of the dense Palade granules. However, very fine dots, smaller than the ribosomes, seem to persist on the surface of the otherwise smooth vesicles, and are apparent in Fig. 9. The membranes of the vesicles appear intact when examined in the electron microscope within the limits of resolution used in this work. The affinity of the vesicles for phosphotungstic acid is unchanged; the material demonstrated inside the vesicles is still present and appears not to be affected by trypsin digestion, even at the highest concentrations of enzyme used (Fig. 8, *g*). The PTA-stained granules (Fig. 8, *ptg*) are also unchanged and remain in their smooth membranous envelope.

FIGURES 8 TO 10

Electron micrographs of washed microsomes incubated for 30 minutes at 37°C with trypsin. Fixation, 1 per cent osmium tetroxide in distilled water. Gelose embedding, followed by methacrylate embedding.

FIGURE 8

Microsome suspension incubated with 40 mg per cent trypsin; staining, phosphotungstic acid. This highest concentration of trypsin used causes the disappearance of the ribosomes from the ring-shaped profiles. As a result, only smooth surfaced membranes persist. The phosphotungstic acid-stained granules (*ptg*), located inside vesicles, have resisted the trypsin treatment. *g*, intravesicular precipitate. $\times 45,000$.

FIGURE 9

Microsome suspension incubated with 2.4 mg per cent trypsin; staining, phosphotungstic acid. Fine dots (*d*), significantly smaller than those observed on untreated microsomes, are present on the outer surface of the microsome vesicles. $\times 45,000$.

FIGURE 10

Microsome suspension incubated with 0.1 mg per cent trypsin; staining, phosphotungstic acid. This concentration of trypsin reduces the size of the ribosomes (*d*). The appearance of the rough vesicle is intermediate between that of trypsin-treated and that of untreated microsomes. $\times 45,000$.

The lowest concentration of trypsin used, 0.15 mg per cent, did not affect the microsomes to the extent of altering the gross appearance of the sediment (Table I). Under the electron microscope, and at relatively low magnification, the general picture is that of untreated microsomes (Fig. 10). On closer examination, however, the dense Palade granules appear to be smaller, *i.e.* 6 to 14 $m\mu$ in diameter instead of the usual 14 to 16 $m\mu$ in the control micrographs (Figs. 1 to 7). Sometimes the membranes appear to show local thickenings in place of granules or fine dots

covered nitrogen of 73, 80, and 62 per cent, respectively, as compared with the 100 per cent for the uncentrifuged suspension. These variable losses suggest that some of the microsomes were disrupted during incubation or resuspension and were not recovered by the centrifugation. In the series of microsome suspensions incubated with trypsin in various proportions the recovery of nitrogen in the pellets fell to about 40 per cent. This finding, and concordant results from many similar experiments, showed that the action of trypsin on washed microsome suspensions was to

TABLE II
Biochemical Composition of the Microsomes

Experimental conditions (Incubations at 37°C for 20 min.)	Pellet (mg)*		Supernatant (mg)*		Pellet: Loss in %†		Supernatant: Gain in %‡	
	Total N	Total P	Total N	Total P	Total N	Total P	Total N	Total P
Experiment 1								
No trypsin	3.05	0.63	1.08	0.098	27	13		
50 mg per cent trypsin	1.77	0.38	2.43	0.28	58	43	44	29
Experiment 2								
No trypsin	3.51	0.61	0.83	0.073	20	12		
3.1 mg per cent trypsin	2.21	0.38	—	—	—	—	—	—
Experiment 3								
No trypsin	2.26	0.37	1.37	0.178	38	33		
0.19 mg per cent trypsin	1.40	0.30	2.03	0.26	60	47	29	23

* The N and P figures for the trypsin-treated microsomes and for the controls (incubated without trypsin) correspond to determinations made on pellets and supernatants. These were obtained after incubation and centrifugation of 2 ml of washed microsomes (stock suspension) to which buffer and eventually trypsin were added.

† The loss in total N and P of the pellet obtained by centrifuging the trypsin-treated microsomes has been calculated in percentages of the N and P determined in both pellet and supernatant.

‡ The gain in total N and P of the supernatant represents the difference between the N and P content of the treated and control supernatants calculated in percentages of the control pellet.

(arrow). This situation seems to be intermediate between that seen in Fig. 9 and that of the normal microsome profiles illustrated in Figs. 1, 3, and 4.

Chemical Observations

The chemical data presented in Table II concern three typical experiments in which three different concentrations of trypsin were used, similar to those in the electron microscope experiments just discussed.

The pellets obtained by centrifugation of the control tubes, *i.e.* those containing the washed microsomes suspended in buffered sucrose and incubated without trypsin, gave values for re-

lower the nitrogen content of the pellet by about 30 per cent; this latter value seemed to be the highest attainable, irrespective of further increase in trypsin concentration, an observation which seems to suggest that the maximum effect, as regards the susceptibility of the microsome substrate to trypsin digestion, had been reached.

The total phosphorus determinations, as shown in Table II, indicate that the loss in total phosphorus of the incubated pellets which may be ascribed to trypsin digestion was 14 and 30 per cent, respectively, for experiments 3 and 1. It must be noted that, in these and in similar experiments not part of the present report, the loss in phos-

phorus of the microsomes subjected to trypsin digestion has, as a rule, been less important and less constant than the loss in total nitrogen, suggesting that the phenomenon is more complex than a simple loosening and removal of the ribosomes from the surface of the microsome vesicles.

DISCUSSION AND CONCLUSIONS

It is now currently admitted that the microsomes isolated and characterized by Claude (6) correspond to preexisting, organized structures of the cytoplasm and cytologically identified under the terms ergastoplasm or endoplasmic reticulum (21, 18). Intensive investigation in the field of biochemistry, and the work of K. R. Porter and of G. E. Palade, have greatly extended the significance of the microsome fraction as obtained by differential centrifugation, and the usefulness of investigations carried out on this fraction *in vitro*.

Palade (19) proposes the following mechanism to explain the transformation of the *in situ* endoplasmic reticulum into microsome vesicles: (a) the endoplasmic reticulum undergoes a fragmentation during the homogenization of the tissue; (b) the broken fragments of the endoplasmic reticulum reconstitute closed vesicles by a "healing" process. Such closed vesicles are sensitive to volume variations when subjected to media of different osmotic concentrations. Under the electron microscope, the microsomes appear as round vesicles covered externally by a layer of dense granules, or ribosomes (19, 20, 16). It thus appears that the ribosomes associated with the original ergastoplasmic membranes are firmly attached to the membrane of the microsome vesicles, since they persist through the relatively severe actions to which they are successively exposed, *i.e.*, homogenizations, sedimentations, resuspensions, and washings. This association is not affected by incubation of the washed microsome suspension for 30 minutes at 37°C, as shown in the present experiments. In the already mentioned investigation of Palade and Siekevitz (19) the same control tests were carried out under the term "aging." A difference observed in the present work concerns the significant increase in the affinity of the microsome vesicles for osmium tetroxide under the effect of incubation at 37°C.

The composition of the microsome pellet is not homogeneous, in the sense that it does not contain a uniform population of the single type of rough, ribosome-covered vesicles. Moulé *et al.* (4) have

succeeded in separating by ultracentrifugation two types of vesicles, rough and smooth, as two sub-fractions. The reason for this is that the original endoplasmic reticulum membranes are not uniform and present smooth, naked profiles, next to rough, ribosome-covered regions; examples of this situation have been described in the case of liver cells, namely, in observations concerned with the distribution of glycogen (8, 9, 15). As a result, at least two types of vesicles, namely the so-called rough and smooth types, may be expected to be found in the microsome fraction. Although this may explain the presence of smooth vesicles in the microsome pellet, a loss of ribosome granules through the process of purifications of the rough vesicles may not be entirely ruled out at this time. Contaminants observed in the purified microsome pellet have been small elements of the microbodies or lysosome series, extensively studied by de Duve and coworkers (10, 11).

In the description of the different types of particles encountered in the microsome fraction, homogeneous granules, 50 m μ in diameter, densely stained with phosphotungstic acid (the PTA-stained granules) have been described. These PTA-stained granules have been constantly encountered in liver cells, as a rule gathered in the neighborhood of bile ducts either singly, or collected in a round, smooth-membraned vesicle. Such PTA-stained granules have been found in greater abundance in the fluffy layer which collects on the top of the microsome pellet in the case of extraction in physiological saline.

The main result registered in the present work has been the apparently specific action of trypsin in the removal of the ribosome elements from the surface of the smooth vesicles. Degrees in the extent of removal of ribosome granules have been observed, depending on the concentration of trypsin. Roth (23) in a previous work on the action of proteolytic enzymes on isolated rat liver microsomes, has pointed out that ribonuclease is present in most of the enzyme preparations and also in the microsome fraction. It is therefore difficult to ascertain the relative importance of the contaminating enzymes in the release of ribosomes (26). The question whether the membrane, itself, of the microsome vesicle has been affected by the trypsin treatment is uncertain, although a greater proportion of nitrogen removed, as compared with that of phosphorus, may suggest that a protein component may have been digested, in addition to the removal of the ribosome particles.

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