trose agar, but development was markedly stimulated by the addition of ethanol. In addition, Raabe (*9*) has reported that aqueous extracts of wood from several plant species will stimulate rhizomorph production in *A. mellea*. Therefore it appears that many natural materials contain a substance or substances which are required by *A. mellea* for rhizomorph development. The fact that alcohols of low molecular weight can replace these complex substrates permits rhizomorphs to be produced on a chemically defined medium so that more precise information on the nutritional requirements of this fungus for rhizomorph production can be obtained.

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24 June 1963

Lysosomes in the Renal Papillae of Rats: Formation Induced by Potassium-Deficient Diet

Abstract. *Lysosomes appeared in the cytoplasm of cells of the renal papillae of rats fed on a potassium-deficient diet. The lysosomes were identified by their morphologic appearance when examined by electron microscopy, and by their acid phosphatase activity shown by both light and electron microscopic examination of Gomori-treated tissue.*

On the basis of biochemical studies of the products of cell fractionation, De Duve postulated that cells must contain organelles in which are localized acid hydrolases, such as acid phosphatase, cathepsin, and beta-glucuronidase (*1, 2*). He gave the name "lysosome" to these particles. Fractions of homogenized cells showing high activity of acid phosphatase, separated by centrifugation, were examined by electron microscopy and found to consist of bodies which were distinguishable from mitochondria, and which had a single limiting membrane (*3*). Since then, examination of sectioned tissue by electron microscopy has shown that bodies having the general morphology just outlined are present in many different kinds of cells (*4–6*). Nonspecific acid phosphatase has been demonstrated in bodies with the structure just described by applying the Gomori method for acid phosphatase to suitably prepared tissues and examining sections by electron microscopy (*5–7*).

Recently, while studying the kidneys of rats made potassium deficient, we recognized that formation and accumulation of lysosomes was taking place in the cells of the renal papilla, where they are not ordinarily found. This observation resulted from our investigation of the characteristic eosinophilic granules, which accumulate in the cytoplasm of renal papillary cells of rats within a week of feeding the animals a potassium-deficient diet (*8, 9*). In previous studies we have shown that the granules contain serum protein and, in addition, a sialic acid–containing mucopolysaccharide (*10, 11*). Since other investigators had already demonstrated histochemically that increased amounts of acid phosphatase accumulate in the cells of the papilla during potassium deficiency in the rat (*12*), it occurred to us that the granules might be lysosomes. This report outlines our evidence that the granules have the biochemical and morphological characteristics recently suggested for lysosomes (*7*).

Our observations were made on male Wistar rats which weighed from 175 to 200 g at the start of the experiment. They were fed a diet low in potassium, obtained from the Nutritional Biochemical Corporation of Cleveland. Control rats were fed the same diet supplemented with potassium salts (0.8 g KH_2PO_4 and 0.6 g KCl per 100 g diet). Each control rat was paired with an experimental animal and given the same daily amount by weight of the control diet as the experimental animal consumed of the low potassium diet. While eating this low potassium diet, rats do not gain weight, but do develop a potassium-deficient state which is characterized by low levels of potassium in the muscles and serum (*10*). Groups of both the experimental and control rats were anesthetized and killed at intervals of 1, 2, 3, 4, and 8 days, and 1 month after the initiation of the diet. Kidneys were removed from each rat through an abdominal incision before killing the animals, and the papilla quickly removed from each kidney.

Table 1. The activities of total, free, and bound acid phosphatase in papillary tissue of normal and potassium-deficient rats. The activities are expressed as micrograms of inorganic phosphorus liberated from β-glycerophospate by 100 mg of renal papillary tissue in 1 hour.

Total	Free	Bound
	Control rats	
65	—	—
82	—	—
66	66	0
77	77	0
119	92	27
107	73	34
100	90	10
75	74	1
*86 ± 7	78 ± 3.4	12 ± 6.6
	Potassium-deficient rats	
709	—	—
750	—	—
453	—	—
488	—	—
798	—	—
417	219	198
332	151	181
431	221	210
840	488	352
684	419	265
645	385	260
401	114	287
290	108	182
*557±52	263±52	242±21

* Mean ± S.E.M.

Papillae for chemical analysis were weighed, and the activity of total tissue acid phosphatase was assayed chemically by the method described by De Duve (*1*); in some papillae the activity of free and bound acid phosphatases was determined also (*1*). Those papillae which were to be examined by light microscopy were fixed in calcium-formol and stained for acid phosphatase by the method of Burstone (*13*). Some of these sections were washed in distilled water after the overnight incubation in Burstone substrate, and then stained by the Hale iron technique for acid mucopolysaccharide following the method of Mowry (*14*).

Tissue for electron microscopic examination was prepared in two ways: by fixation in osmium tetroxide, and by the method of Miller for acid phosphatase localization (*6*). All solutions for Miller's enzyme technique were made up with 0.35*M* sucrose, rather than the 0.22*M* sucrose suggested by

Miller. The higher concentration of sucrose gave better preservation of tissue structure.

Chemical assay of acid phosphatase activity of the renal papilla was determined in rats fed the experimental diet for 4 weeks. The results are shown in Table 1. The total acid phosphatase activity of papillae from the potassium-deficient rats was six or seven times greater than that of the controls. In those animals in which the free and bound acid phosphatase activities were also determined, the increase in bound phosphatase was relatively greater than that of the free phosphatase in the potassium-deficient rats. This rise in bound acid phosphatase would be expected were there an accumulation of lysosomes containing the enzyme in the renal papillary cells of the potassium-deficient rats.

The cells of renal papillae from rats fed the experimental diet for 4 weeks showed abundant Hale-positive granules. No such granules were seen in the papillae of the control rats. Sections stained by the Burstone method showed acid phosphatase activity in cells of the papillae of the potassium-deficient rats. The reaction product of enzyme activity was most prominent in those granules seen in interstitial cells of the papillae and in the endothelial cells of the vasa recta, although it was present in a moderate amount in the cell granules of the collecting tubules as well. When both acid phosphatase stain and Hale iron stain were applied to the same sections, it was noted that acid phosphatase and Hale-positive material were localized in the same granule. This was distinct in the granules present in interstitial cells, but was not so evident in the granules of collecting tubule cells. Our electron microscopic studies permitted us to locate more precisely the acid phosphatase activity in the characteristic granules of both collecting tubule and interstitial cells. The ultrastructure of the collecting tubule and other cells of the potassium-deficient rat was similar in appearance to those of the same type described by others (*9*) (see Figs. 1 and 2). Large numbers of typical droplets or granules were visible by the third day after feeding the low potassium diet. These ranged in size from 0.5 to 4.0 μ. Mitochondria retained their characteristic morphology, and since they are present only in small numbers in collecting tubule cells of the renal papillae, it is unlikely that there was any confusion between them and the very numerous characteristic granules. We noted further that the granules were variable in composition, and some contained microvesicles of 100 to 300 Å, while others showed homogeneous dense material. Composites of the two types of granules were also frequent. A single type of limiting membrane surrounded most of the bodies. In some bodies the membrane was incomplete, and in a few of the multivesicular-type bodies it was absent.

In the renal papillary tissue shown in Fig. 3, the crystalline lead phosphate, produced by the Gomori acid phosphatase technique, is seen deposited in the granules. The reaction product is not seen in all granules, but no lead phosphate is seen in other cell structures.

These observations show that structures which are morphologically, biochemically, and electron microhistochemically indistinguishable from lysosomes, appear in the cytoplasm of renal papillary cells of rats made potassium-deficient. Furthermore, these structures arise in cells where they are either not detected in the normal animal or are present in extremely low numbers. Potassium deficiency in rats thus offers a means for the experimental induction of lysosome formation and for further study of the origin of these interesting bodies (*15*).

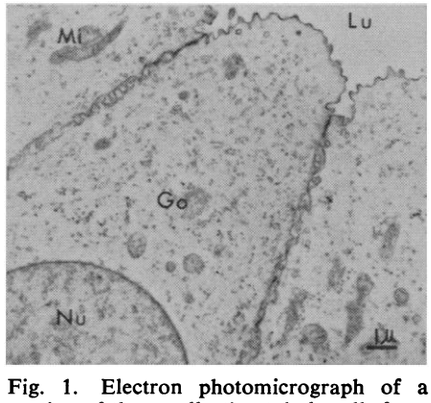
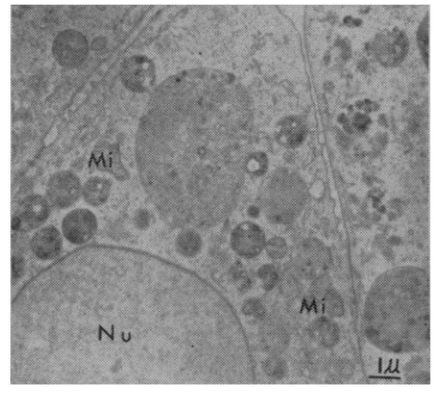


Fig. 1. Electron photomicrograph of a portion of three collecting tubule cells from the renal papilla of a normal rat. The short microvilli project into the tubular lumen (Lu). The cytoplasm contains numerous tiny vesicles, prominent Golgi apparatus (Go), and a few mitochondria (Mi); nucleus (Nu). Tissue fixed in osmium-tetroxide and sections stained by the lead method of Karnovsky (*16*) (about × 9700).



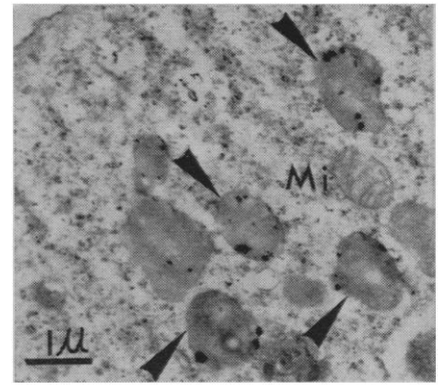


Fig. 2 (left). Electron photomicrograph of a portion of three collecting tubule cells from the renal papilla of a potassium-deficient rat. Numerous lysosomes of varying configuration are present. A few mitochondria (Mi) are easily distinguished from the other cellular structures. Tissue prepared as in Fig. 1 (about × 9700). Fig. 3 (right). Electron photomicrograph of portion of collecting tubule cell from a potassium-deficient rat. The tissue was prepared for electron microscopy after localization of acid phosphatase by the Gomori technique. Arrows indicate lysosomes showing deposition of crystalline lead phosphate which defines foci of acid phosphatase activity. A mitochondrion (Mi) can be clearly distinguished and shows no lead phosphate deposit (about × 10,000).

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29 August 1963

Pea Aphid: Rearing on a Chemically Defined Diet

Abstract. *Pea aphids,* Acyrthosiphon pisum *(Harr.), reared for two successive generations on a diet made of 23 amino acids and amides, 11 water-soluble vitamins, 35 percent sucrose, salts, and cholesterol, remained apterous. They grew and developed during the first generation almost as well as those grown on pea plants, with very little mortality.*

Information on the rearing of aphids on synthetic diets is very limited. Various attempts to feed these insects on gels or liquids, sometimes under pressure, and with the use of various types of membranes, usually ended in failure, the insects surviving for about 1 week (*1*). Recently, a first limited but significant success was reported (*2*) on the artificial feeding and rearing of an aphid (*Myzus persicae*) on a chemically defined diet. Although high mortality occurred, a few nymphs transformed into diminutive adults after 16 to 17 days of rearing. This diet, when fed to the pea aphid, *Acyrthosiphon pisum* (Harr.), supported little growth and resulted in the death of all nymphs within 1 week. During the past year, we have developed a chemical diet which gives pea aphid growth rates and development almost equivalent to those obtained on pea plants, with almost no mortality during the first generation.

The type of cage used is similar to one already described (*3*), except that it is larger and better ventilated; the plaster of Paris in the bottom is replaced by a wet cotton wad, and the aphids are further confined within an inverted cone of nylon. The aphids have access to the liquid diet by way of a natural rubber (*3*) or a Parafilm (*2, 4*) membrane which they "puncture" to feed. One of the diets that has so far given satisfactory results is as follows.

L-Amino acids and amides (mg): alanine, 100; arginine, 400; asparagine, 300; aspartic acid, 100; cysteine, 50; cystine, 5; γ-amino butyric acid, 20; glutamic acid, 200; glutamine, 600; glycine, 20; histidine, 200; isoleucine, 200; leucine, 200; lysine mono hydrochloride, 200; methionine, 100; phenylalanine, 100; proline, 100; serine, 100; threonine, 200; tryptophan, 100; tyrosine, 20; valine, 200; and DL homoserine, 800 mg to make a total of 4315 mg of amino acids and derivatives. The diet also contained the following vitamins (mg): ascorbic acid, 10.0; biotin, 0.1; calcium pantothenate, 5.0; choline chloride, 50.0; folic acid, 1.0; i-inositol, 50.0; nicotinic acid, 10.0; p-aminobenzoic acid, 10.0; pyridoxine hydrochloride, 2.5; riboflavin, 5.0; and thiamine hydrochloride, 2.5, to make a total of 146.1 mg. In addition the following were added, cholesterol benzoate, 2.5 mg; K_3PO_4, 500.0 mg; $MgCl_2 \cdot 6H_2O$, 200.0 mg; salt mixture No. 2 USP, 5.0 mg; sucrose, 35.0 g; water (to make) 100.0 ml; at *p*H, 7.3 to 7.4.

The choice and quantity of each amino acid and amide in the diet was based on the average concentration of these compounds in pea-aphid blood and honeydew (*5*), but the diet contained twice the concentrations measured. Arginine, cysteine, histidine, lysine, and tryptophan, not reported in pea-aphid blood and honeydew, were added to the diet. The vitamins and salt mixtures described (diet I) were, with slight modifications and additions, similar to those used for *Myzus persicae* (*2*). From this diet I, a diet II was prepared by reducing the total concentration of amino compounds and vitamins from 4.46 to 2.12 percent. In addition, a diet IV was prepared identical to that used for *Myzus persicae* (*2*) containing 18 percent sucrose and 2.25 percent amino compounds and vitamins; finally, diet III was prepared identical to diet IV, but its sucrose content was raised to 35 percent. The addition of the salts resulted in slight turbidity, especially in diet I, and was probably due to the formation of small amounts of poorly soluble magnesium phosphate. Contamination by microorganisms was minimized by pasteurizing diets and renewing them in the feeding cages every 4 to 5 days. Membranes were dipped for a few minutes into 70 percent ethanol, dried, and used immediately thereafter.

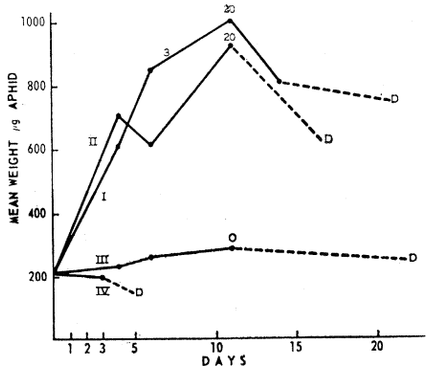

Fig. 1. Growth curves of nymphs of pea aphids reared from first-instar on four chemical diets (I–IV). The arabic numbers above each curve indicate the number of adults produced out of 20 nymphs. *D* = death of the last aphid.

The four diets were fed to groups of 40 first-instar nymphs of pea aphids averaging 215 µg per nymph; nymphs were maintained at 20°C, high humidity, and under a 16-hour photoperiod. On the 7th day of the test, live aphids on diets I, II, and III numbered respectively 36, 35, and 28; the numbers were further reduced to 20 aphids per diet. The results on growth and development (Fig. 1) show that all nymphs on diets I and II reached the adult stage by the 11th day of feeding, increasing their weight by 333 to 450 percent. None of the nymphs on diets III and IV reached the adult stage, and the maximum average weight gain on diet III was only 36 percent. Adults reared from first-instar nymphs on diets I and II gave birth to numerous nymphs and then died after 17 to 21 days of feeding. On other similar diets, some aphids survived for as long as 30 days; on pea plants, they usually died 30 to 40 days after birth. In another test, the first 30 nymphs born from aphids reared on diet I from first instar to the adult stage were also kept on the same diet. At birth, these second-generation nymphs weighed 60 to 70 µg each, and many of them transformed after 17 days into small second-generation adults weighing 450 to 500 µg each, thus representing an average of 630 percent weight increase. It is also significant that the test nymphs, obtained from a pea-aphid culture and capable of producing winged forms under certain conditions, remained apterous during two generations on the artificial diet. Furthermore, aphids feeding on diet I almost always remained immobile and apparently well "satisfied," even though no substances were