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REVIEW ARTICLE

Insulin Secretion: Multifactorial Regulation for a Single Process of Release

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Since the work of Minkowski [1], it is known that, in order to maintain normoglycemia, the mammalian organism is dependent on a continuous and continuously adjusted supply of pancreatic insulin. A dramatic illustration of such a permanent need for insulin is given by the hyperglycemia seen in normal animals injected with guinea-pig anti-insulin serum [2]. In these animals, the rate of endogenous insulin secretion can be calculated from the progressive reduction in the pool of circulating unneutralized antibodies [3]. A similar method has been used to measure insulin secretion *in vitro* from incubated pieces of rat pancreatic tissue [4]. Using this procedure, it was possible to investigate the influence upon the beta cell of a number of metabolic, hormonal and neurohumoral factors possibly involved in the fine regulation of insulin secretion *in vivo* [5]. In the present lecture, we wish to discuss the significance of and interrelationship between the insulinotropic effect of these various agents.

Glucose-simulating and glucose-potentiating agents

The secretory response of the beta cell is characterized by its rapidity and sensitivity [6]. The latter attribute is illustrated by the sigmoidal relationship between the rate of insulin release and the glucose concentration of the medium bathing the beta cell [4]. Because of this sigmoidal relationship, it is not possible to adequately characterize the insulinotropic effect of any given agent, unless the influence of this agent is systematically tested at all glucose levels [7]. When such a systematic investigation is carried out, two fundamentally different types of behaviour are observed, as shown in Fig. 1.

In the upper and left panel of Fig. 1, the heavy line represents the normal sigmoidal relationship between insulin output and glucose concentration. The other

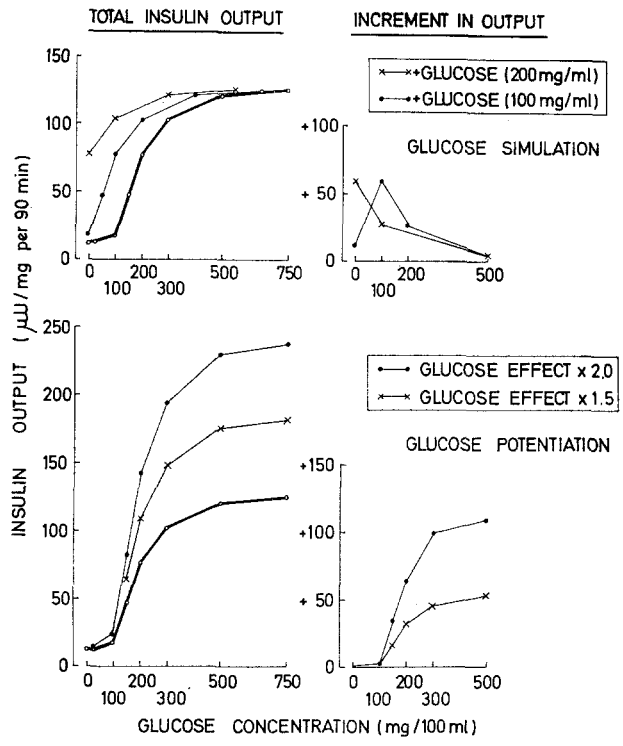

Fig. 1. Definition of glucose-simulating (upper panel) and glucose-potentiating (lower panel) insulinotropic agents. The total insulin output (left) and the increment in secretion rate (right) evoked by these agents in pieces of rat pancreatic tissue are shown as a function of the glucose concentration of the incubation medium (indicated along the abscissa). On the left, the rate of insulin output induced by glucose alone is shown by the heavy line (see ref. 4). The other curves were derived from the experimental data, as shown in the rectangles, by assuming either that a fixed amount of glucose (1.0 or 2.0 mg/ml) was added to the glucose already present in the incubation medium (glucose simulation), or that the insulinotropic action of glucose was multiplied by a constant factor (× 1.5 or 2.0; glucose potentiation)

curves indicate what would happen to the rate of secretion if a given insulinotropic agent added to the incubation medium were to lower the "K_m" without affecting the "V_{max}" of the secretory mechanism. Since these curves were obtained by assuming that a fixed and additive amount of glucose is added to the glucose already present in the incubation medium, we propose to qualify any insulinotropic factor acting in such a manner as a *glucose-simulating* agent. It is shown in the upper and right panel of Fig. 1 that the absolute increment in secretion rate induced by glucose-simulating agents is maximal either in the absence of glucose or at intermediate glucose levels and always fades out at the highest glucose concentrations.

The second type of behaviour is shown in the lower part of Fig. 1. It is assumed that the agents under study do not affect the "K_m", but increase the "V_{max}" of the secretory process, the stimulant action of glucose being multiplied by a constant factor. With such agents, to which we will refer as *glucose-potentiating* agents, the absolute increment in secretion rate, in excess of that induced by glucose alone, is null in the absence of glucose and maximal at the highest glucose concentration.

We have shown elsewhere [7] that the first type of behaviour, i.e. the glucose-simulating effect, is characteristic of the metabolic regulation of insulin release by various substrates including glucose, other sugars, certain amino acids such as leucine, and different lipid metabolites [8, 9, 10]; whereas the second type of behaviour, i.e. the glucose-potentiating effect, is characteristic of the hormonal modulation of insulin secretion by adrenergic and polypeptidic factors acting on the beta cell through its adenylcyclase system [11, 12, 13]. We now wish to emphasize that these two different behaviours correspond to fundamentally different mechanisms, not only in terms of the physiological regulation of insulin secretion by environmental factors but also in terms of the underlying molecular process in the beta cell.

The intimate mode of action in the beta cell of glucose-simulating agents

Because glucose undoubtedly represents the physiologically most important insulinotropic agent among the glucose-simulating metabolites, its intimate mode of action in the beta cell will be considered here in some detail. One of the most controversial issues in this respect concerns the nature of the signal in glucose-induced insulin release. We would still favour the idea that glucose has to be transported and metabolized in the beta cell in order to stimulate insulin secretion. This belief is naively based on the specific inhibition of glucose-induced insulin release, as distinct from leucine- or sulfonylurea-induced secretion, by agents, such as mannoheptulose, known to alter the metabolism of glucose in the beta cell [10, 14, 15]. There is also an ample body of evidence indicating that the process of insulin release requires energy, so that the production of ATP has to be considered, at least, as a permissive factor [4, 16]. These matters are not discussed here in greater detail, as we wish to concentrate on the next event in the secretory sequence, namely that which links the recognition of glucose to the release of insulin. Because of the well known dependency of insulin secretion on extracellular calcium [13, 17, 18], we have hypothesized that such a link might be an accumulation of calcium in the beta cell [19].

A first observation in favour of such a concept is the fact that glucose stimulates the net uptake of radioactivity by isolated islets incubated for 90 min in the presence of ^{45}calcium and submitted thereafter to repeated washes in order to remove extracellular ^{45}calcium [20]. This procedure, however, does not yield any information concerning the rapid changes in calcium metabolism which might occur on exposure of the beta cell to glucose.

In order to investigate the dynamics of calcium handling by the beta cell, isolated islets were preincubated in the presence of ^{45}calcium and then placed in a small perifusion chamber, so that the efflux of ^{45}calcium from the islets could be continuously monitored. The ^{45}calcium recovered in the effluent media apparently consists of two fractions (Fig. 2). The first fraction is thought to represent ^{45}calcium transported across the intact membrane of the beta cell. The second

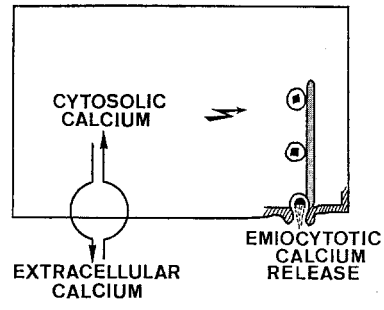

Fig. 2. Schematic representation of the exchange of calcium between the cytosol and the interstitial fluid, across the plasma membrane of the beta cell (left); and for the release of calcium occuring at the time and site of emiocytosis (right). The forked arrow depicts the activation of the microtubular-microfilamentous system by cytosolic calcium

fraction is thought to correspond to a release of ^{45}calcium occuring at the time and site of emiocytosis; for instance, it might represent ^{45}calcium previously enclosed within the membranous sacs surrounding the secretory granules [21].

As shown in Fig. 3 (left panel), when the glucose concentration of the perifusate was increased from zero to 3.0 mg/ml, an immediate reduction in ^{45}calcium efflux was observed, suggesting that glucose inhibits the transport of calcium from the cytosol into the interstitial fluid. This initial fall was followed by a

dramatic secondary increase in ^{45}calcium efflux which occurred concomitantly to the onset of glucose-induced insulin secretion and probably corresponds to the emiocytotic release of ^{45}calcium. When the experiments were repeated in media deprived of extracellular calcium (Fig. 3, right panel), thus under a condition known to abolish insulin release, glucose provoked an immediate and marked reduction in ^{45}calcium outward transport across the cell membrane, but no secondary rise was observed.

An important implication of the present model is that, if the amount of calcium accumulated in the beta cell indeed controls the rate of insulin secretion, there should exist an invariable, universal and obligatory function relating insulin output to the net uptake of calcium by the beta cell [23]. As reported in detail elsewhere, a relationship between the rate of insulin release and the amount of ^{45}calcium accumulated in the beta cell was indeed observed, when the effect of glucose was examined in the presence or

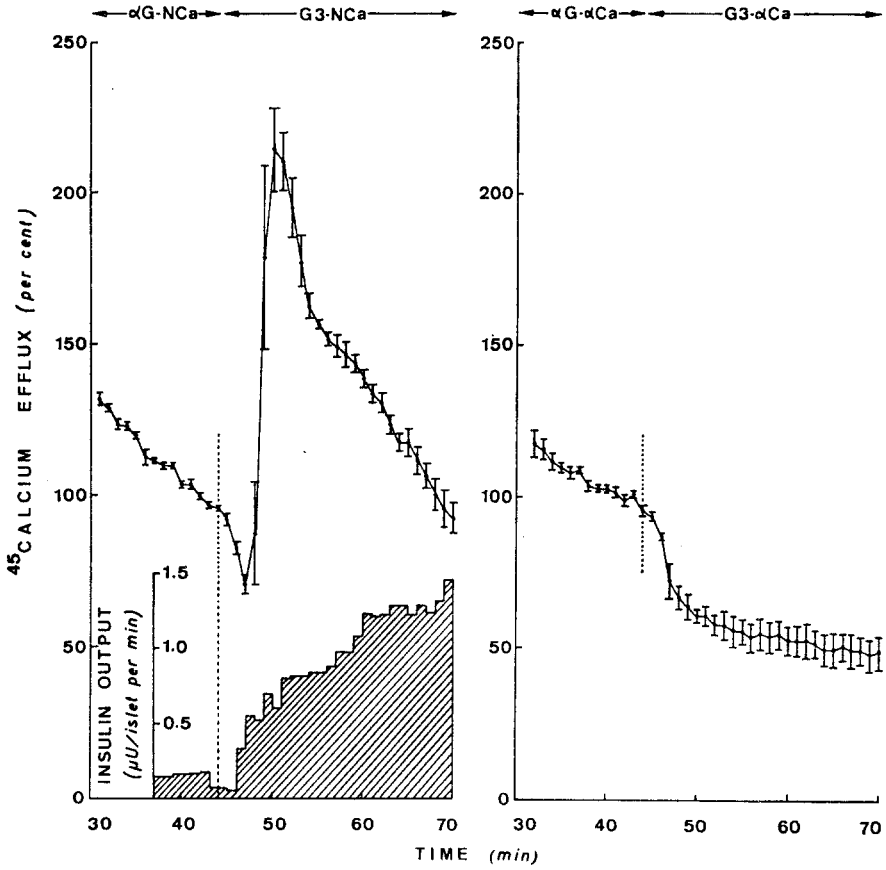

Fig. 3. Mean values (± SEM) for ^{45}calcium efflux from rat islets perifused up to the 44th min in the absence of glucose (αG), and from the 45th to 70th min at a glucose concentration of 3.0 mg/ml (G3). The experiments were performed either at "normal" calcium concentration (NCa; left panel), or in the absence of extracellular calcium (αCa; right panel). Within each individual experiment, the efflux of ^{45}calcium (cpm/min) was expressed in per cent of the mean value observed between the 40th and 44th min. Also shown is the mean insulin output by the islets. The dotted vertical line corresponds to the time at which glucose was added to the perifusate

The findings illustrated in Fig. 3 suggest that glucose, by inhibiting the outward transport of calcium, provokes an intracellular accumulation of calcium, which in turn triggers insulin release. We have investigated the mechanism of such a glucose-induced accumulation of calcium in the beta cell. Our tentative conclusions are that it represents an active, energy-consuming process, dependent on the integrity of glucose metabolism in the beta cell, and mediated through the activation of a calcium-dependent sodium pump located at the cell membrane [20, 22].

absence of various metabolic, ionic and pharmacological factors [20]. Moreover, an identical relationship between insulin output and calcium uptake was found when the effect of other glucose-simulating agents, such as mannose and leucine, was investigated [24].

The influence of glucose-potentiating agents on calcium handling by the beta cell

The only obvious exception to the above-mentioned invariable relationship between the net uptake of

^{45}calcium and the subsequent release of insulin by isolated islets was encountered when the effect of dibutyryl-cAMP (db-cAMP) or theophylline was investigated. Thus, these agents markedly enhanced glucose-induced insulin release, without affecting glucose-induced ^{45}calcium uptake [25]. It is remarkable that this apparent exception was observed with insulinotropic drugs which behave as typical glucose-potentiating agents. Indeed, all agents known to increase the level of cAMP in the beta cell — including glucagon, enteroglucagon, db-cAMP, cAMP, theophylline or caffeine — are unable to provoke sustained secretion of insulin in the absence of glucose, and exert their most marked insulinotropic action at high glucose levels [11, 12, 13].

The fact that these agents do not modify the stimulant action of glucose on ^{45}calcium uptake does not necessarily rule out our working hypothesis that the level of calcium in the cytosol of the beta cell regulates the rate of insulin secretion. It is indeed conceivable that these agents, instead of acting primarily upon calcium transport across the cell membrane, provoke an intracellular translocation of calcium from an organelle-bound pool (e.g. the vacuolar system known to be actively involved in the subcellular distribution of calcium) into the cytosol (Fig. 4).

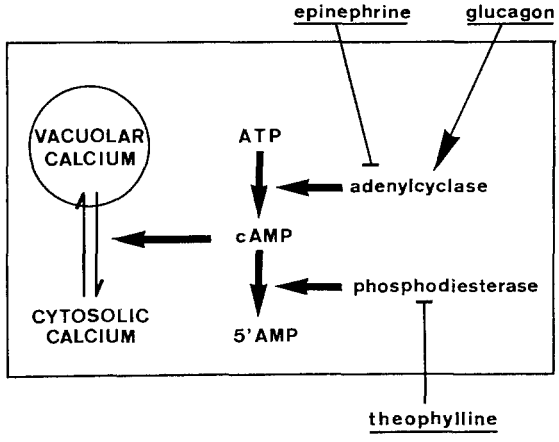

Fig. 4. Hypothetical model for the effect of glucose-potentiating agents upon calcium metabolism in the beta cell. It is postulated that an increase in the level of cyclic AMP provokes an intracellular translocation of calcium from the vacuolar system into the cytosol. The activation or inhibition of enzymes involved in cyclic AMP metabolism is indicated by arrows and T-shaped bars, respectively

The following observations are quoted in favour of such a concept.

First, glucose or leucine, which are unable to stimulate insulin secretion in the absence of extracellular calcium, exert a definite insulinotropic action when the calcium-depleted media also contain either db-cAMP or theophylline, suggesting that these agents modify the internal milieu of the beta cell in such a way that the normal supply of extracellular calcium is no more required for glucose or leucine to provoke insulin release [25].

Second, theophylline provokes an immediate and sustained increase in ^{45}calcium efflux from perifused islets, suggesting a sudden enrichment of the pool of cytosolic calcium readily available for outward transport across the cell membrane [25, 26]. The theophylline-induced increase in ^{45}calcium efflux is much more marked in the absence than in the presence of glucose [26]. This observation suggests that, in the absence of glucose, most if not all of the calcium load initially translocated in the cytosol escapes from the beta cell, a process which would account for the failure of theophylline alone to stimulate insulin release; whereas, when glucose is also present, the sugar might, by inhibiting the outward transport of calcium across the cell membrane, retain most of the translocated calcium intracellularly and, in doing so, allow theophylline to exert its glucose-potentiating insulinotropic action [26].

In summary, it appears that glucose-potentiating agents, which also share in common the property of increasing cAMP levels in the beta cell [12], provoke an intracellular shift of calcium from the vacuolar system in the cytosol. However, such a shift will only cause a sustained increase in insulin output if the beta cell is simultaneously exposed to a glucose-simulating agent (e.g. glucose or leucine), which itself prevents the load of calcium initially translocated in the cytosol to escape from the beta cell.

The participation of a microtubular-microfilamentous system in insulin release

Since the above-mentioned findings converge in the idea that the level of calcium in the cytosol regulates the rate of insulin secretion, the question now to be considered is that of the site of action of calcium in the secretory sequence. Based on the analogy between stimulus-secretion coupling in the beta cell and excitation-contraction coupling in muscle, we have postulated that calcium could trigger insulin release by activating a contractile microtubular-microfilamentous system involved in the migration and extrusion of secretory granules [19]. This system, first recognized in the beta cell by Lacy *et al.* [27] is schematically depicted in Fig. 5.

Ultrastructural studies by Malaisse-Lagae *et al.* [28] and Orci *et al.* [29] have led to the suggestion that secretory granules could be guided to the cell surface by a cytoskeleton composed of microtubules. Thus, microtubules can be found in close vicinity to secretory granules, and, occasionally, are ending in and intermingled with the microfilamentous cell web. This cell web is formed of a network of microfilaments, disposed either in parallel arrangement or as irregularly shaped polygons, just beneath the cell membrane. This ectoplasmic band could well control the access of

secretory granules to the cell membrane and their extrusion in the process of emiocytosis.

In order to assess the participation of such a microtubular-microfilamentous system in the process of insulin secretion, we have investigated the influence upon the beta cell of a series of drugs known to interfere with the function and structure of microtubules and microfilaments. Briefly, the following results were obtained. Mitotic spindle inhibitors such as colchicine and vincristine were found to cause the disappearance of microtubules and to inhibit insulin secretion in response to glucose or the combination of glucose and theophylline [25, 28, 30]. In addition, vincristine provokes the massive precipitation of crystalline-like material, presumably derived from the microtubular protein. These ultrastructural changes in the beta cell and the inhibition of insulin release follow a synchronous pattern during exposure to vincristine. Deuterium oxide, which overstabilizes the microtubules in the beta cell, also inhibits glucose-, leucine- and sulfonylurea-induced insulin release [24, 28, 30, 31]. Cytochalasin B, which causes a spatial redistribution of the cell web with the appearance of large masses of microfilamentous material, a shortening of microvillous processes and the margination of secretory granules, facilitates insulin release evoked by glucose, sulfonylurea or the combination of glucose and theophylline (29, 32]. It should be noted that neither vincristine, nor heavy water, nor cytochalasin B have any obvious effect upon glucose-induced ^{45}calcium accumulation in the beta cell, thus supporting the concept that these agents affect insulin release by altering the response of the microtubular-microfilamentous system to the triggering action of calcium, as reviewed in detail elsewhere [23].

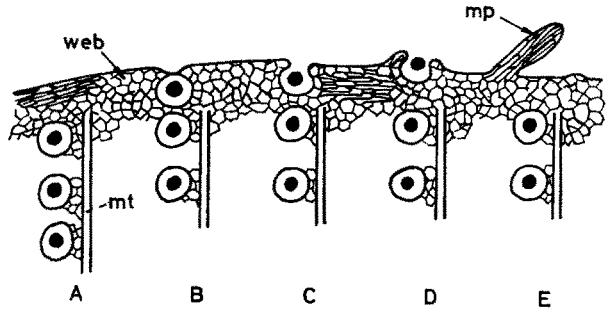

Fig. 5. Schematic representation of the relationship between secretory granules, microtubules (mt), and the microfilamentous cell web. In the unstimulated beta cell, the secretory granules are kept away from the plasma membrane by the cell web (A). Under stimulation, granules are transported along the microtubules, and the web might participate in their access to the cell membrane (B). After fusion between the granule membrane and the plasma membrane, an emiocytotic aperture occurs (C), and the granule core is extruded into the extracellular space (D). The incorporation of the membranous sacs encasing the granules into the plasma membrane apparently results in the formation of microvillous processes (mp; E)

In summary, microtubules are apparently required for the transport of secretory granules to the cell web, which itself might act as a barrier controlling the contact of the granules with the plasma membrane. In a more recent study, we have investigated whether the microtubular-microfilamentous system also participates in the immediate secretory response of the isolated perfused rat pancreas to glucose and other insulinotropic agents [33]. This work is still in progress. The data so far obtained indicate that microtubules and microfilaments indeed play an integral role in both the early and late phases of insulin secretion.

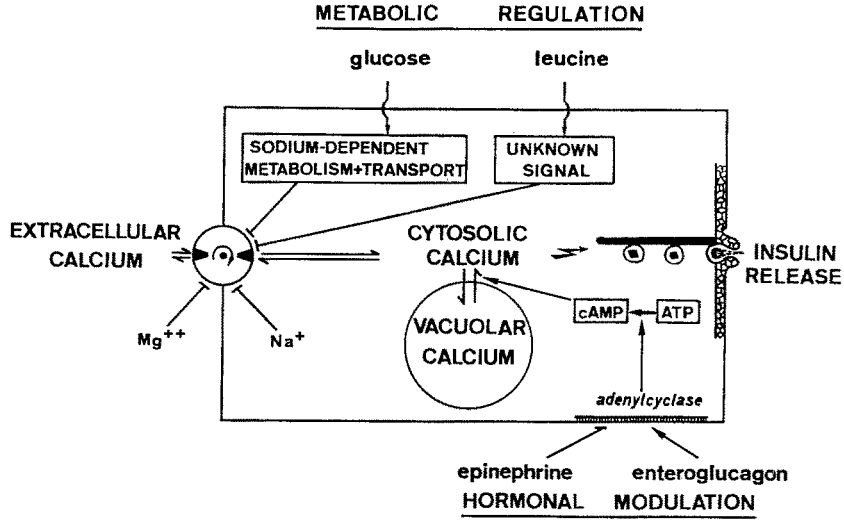

Fig. 6. An integrated model for the multifactorial regulation of insulin secretion by metabolic and hormonal agents. On the left side, the T-shaped bars represent the hypothetical sites of competition or inhibition of calcium transport across the cell membrane. The other symbols are identical to those used in Fig. 2 and 4

The participation of the microtubular-microfilamentous system in the dynamics of insulin secretion

might have pathophysiological implications. Thus, Malaisse-Lagae *et al.* [34] have recently reported that the amount of microtubular material in the beta cell of spiny mice is much lower than that found in the beta cell of other rodents. Since spiny mice are characterized by active insulin biosynthesis and storage in the beta cell, but a sluggish secretory response of the pancreas to various stimuli [35, 36], it is tempting to speculate that the diabetic syndrome found in these animals could be due, in part at least, to an abnormality of the microtubular system, rendering the beta cell unable to mobilize its stores of hormone.

Conclusion

Fig. 6 summarizes our presentation in this lecture, and aims at illustrating how the multifactorial regulation of insulin secretion by circulating nutrients and hormonal agents might eventually be mediated through a single process of release, which involves the level of calcium in the cytosol of the beta cell and the subsequent activation of the microtubular-microfilamentous system. Although the model depicted in this figure should still be considered as a working hypothesis, it is nevertheless compatible with the experimental data so far available.

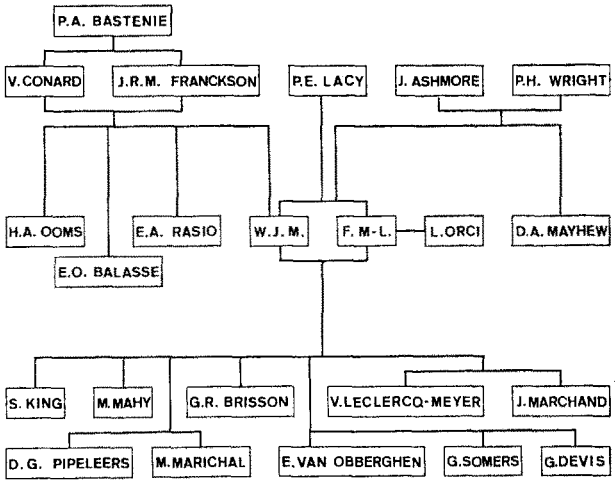

Fig. 7. The 1972 Minkowski awardees

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