In our experiments, the doses of eledoisin which produced an initial fall in arterial blood pressure equal to that induced by 1 μg/kg of bradykinin varied from 0.024 to 0.066 μg/kg.

The Table indicates the equivalence of doses of bradykinin and eledoisin before and after administration of BPF in 4 different dogs. The ratios of the activity of eledoisin/bradykinin, which varied from 15–41 in the control experiments, were reduced to almost equivalency ranging from 1.0 to 2.0 after treatment with BPF. It is to be noted that the doses of bradykinin (0.024–0.066 μg/kg), which produced noticeable effects after treatment with BPF, were below the threshold doses necessary to produce any effect before the treatment. Kallidin showed a behaviour similar to that of bradykinin, being strongly potentiated by BPF.

In conclusion, the results presented in this communication show that the pharmacological potency of bradykinin on guinea-pig ileum and arterial blood pressure of the cat and the dog becomes similar or stronger than that of eledoisin after BPF treatment.

Our present findings give further support to the suggestion[2,3] that bradykinin potentiation by BPF could be due to the blockade of the peptide inactivation by blood, or by kininases possibly present in the smooth muscles, at sites close to the pharmacological receptors[10].

Zusammenfassung. Nachweis der pharmakologischen Wirkung des Bradykinins am isolierten Meerschweinchenileum und am deutlichen Blutdruckanstieg bei Hund und Katze nach Vorbehandlung mit BPF (Bradykininpotenzierender Faktor aus Jararacagift). Unter diesen Bedingungen werden mit Bradykinin gleich intensive Effekte wie mit Eledoisin erhalten.

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COGITATIONES

Mitotic Rate in Organs and Tissues in Relation to Metabolic Body Size ($kg^{3/4}$)

On the molecular level of metabolism, the concentration of certain respiratory enzymes, such as cytochrome-*c*, cytochrome oxidase, etc., per unit of metabolically active mass ($kg^{¾}$) is proportional to overall energy exchange per kg – for rats, dogs, men and cows[1,2]. At least in mammalian liver, the amount of mitochondria bears the same quantitative relation to total metabolism as to total body size, and it is likely that the relative amounts of these elements in any given tissue will prove to be the controlling factor in determining the regression of oxygen utilization on total body size of the species[3,4].

In a broad and metabolic sense, cellular life-span is closely correlated with the life-span of certain cellular components or metabolites. Thus, the $\tau_{½}$ – half time – of cytochrome-*c* in liver may be as representative of the turnover time of the liver cell as is the $\tau_{½}$ of hemoglobin of that of the red blood cell[5].

Metabolic processes in the rat, for example, go at rates some 4.6 times faster than those in man. The metabolic rate factor 4.6 reflects the degree of difference between the adult rat and adult man in rate per unit time and per unit mass of such measurable quantities as the basal metabolic rate (i.e. oxygen consumption), the outflow of nitrogen in the urine, or of protein production[5].

The mitochondrial energy transduction in aerobic organisms is based on citric acid cycle oxidations and fatty acid oxidation, coupling electron flow to synthesis of adenosine triphosphate (ATP), that is, oxidative phosphorylation. The components of the electron transport system are currently though of as consisting of a series of physically interconnected lipoproteins with electron acceptors (coenzymes) tightly bound. The terminal catalyst appears to be cytochrome oxidase and may be the *rate-limiting factor.*

Other studies, although indirectly, substantiate the function of cytochrome oxidase as a rate-limiting factor. Chance's work[6] indicates that in the intact mitochondria, the cytochrome oxidase velocity constant is 4 to 23-fold less than that of the other reactions. Another item of evidence is that the absolute values of the total cytochrome oxidase activity in certain large as well as small animals were found to be very nearly the same as the maximal metabolism of the intact animal[7].

With cytochrome oxidase as a rate-limiting factor, increasing body size is accompanied by decreasing respiratory intensity – in analogy, a small clock must have a faster-swinging pendulum than a large one – in vivo and in vitro[8], and the duration of the metabolic process, Life,

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[8] As well as by decreasing mitochondrial ribonucleic acid content[9]. Smaller body space (organism size) of an animal requires a higher intensity of body time (shorter chronological time) to live. Similar relations exist on the organ level; compare, for example, the interdependence between heart size and beating frequency[10].
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can be conveniently expressed in terms of oxygen consumption (QO_2) per unit body surface. It becomes apparent that all animals, large or small, homeothermic or poikilothermic, burn the light of their lives with relative equality. Life, at least on the organismic level, is a democratic process: all of us must die, and the duration of our existence is the same – if measured in QO_2 per unit body surface[11].

In re-examining the relation between life-span and metabolic rate it can be concluded that women burn their fire of life at an approximately 10% lower rate, are in general smaller – more closely resembling a spherical surface – than men, and have a correspondingly lower age specific death rate than men at *all* adult age levels[11]. There is good evidence in the literature to support such a contention. SACHER[12], for example, concludes that the mortality rate of different species and the rate of increase in mortality with age in the same species are positively correlated. This correlation forms a possible basis for the relative constancy in his life table for species as widely differing as the fruit-fly, the mouse, and man. In 1959, SACHER[13] described a statistical analysis of the relation of life-span to body weight for 63 species of mammals. He established the quantitative dependence of the life-spans of these species on body weights, brain weights and metabolic rates of adult representatives of the species. He regarded the life-span as a physical dimension of a species on the same footing as linear or mass dimensions. In reformulating Rubner's theory of aging, he states that the life-span of a species varies inversely as its basal metabolic rate.

In summary: metabolically active body size ($kg^{3/4}$) and life-span are interrelated phenomena.

How may we, then, conceive of the change in the number of cells, i.e. the growing (aging) of individual tissues and organs within an organism, of an open system in (homeostatic) equilibrium? Evidently not all tissues and organs grow (age) simultaneously at the same rate.

We know that genetic information specifies the leaf form of wheat independent of genetic influences on cell sizes, shapes and on extents and orientations of cell divisions[14].

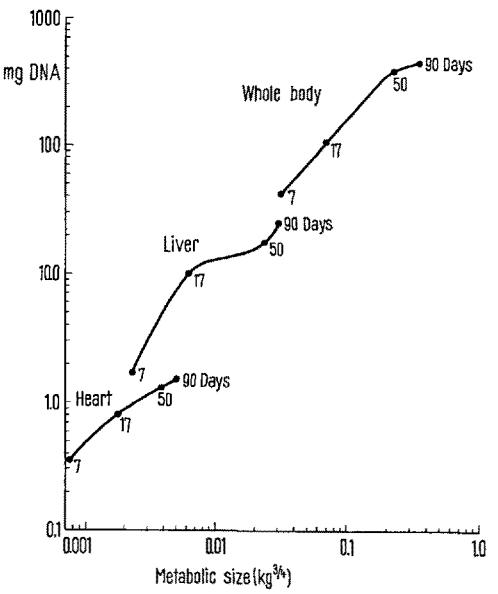
Another related problem is that organs having cells which seldom, if ever, divide have no opportunity to throw off either spontaneous or induced mutations, and it is these organs which are responsible for the aging of the animal[15]. Thus, such tissues as bone marrow show no aging, whereas tissues with non-dividing cells such as brain are responsible for the aging of the whole organism[16]. Let it be recalled that SACHER's correlation between mammalian life-span and brain weight strongly supported the hypothesis that an important determining factor of life-span is the precision of physiologic regulation[17].

For SOROKIN[18], who distinguishes two periods during the development of a cell – one from the origin of the cell to the beginning of differentiation, another from the start of differentiation to the death of the cell – aging is a developmental aspect of metabolism.

Since the precision of the metabolic regulatory process is contained in the relation between metabolically active body size ($kg^{3/4}$) and life-span, the age dependent mitotic rate of tissues and organs should be specified in relation to metabolically active body size. A plot of the above relation has not yet been presented in the literature.

ENESCO and LEBLOND[19] recently determined the pattern of change in the number of cells in a series of organs and tissues – of the growing rat – as the organs and tissues increased in size. Weight and DNA content of the body and of organs and tissues of the male rat at various ages were recorded. Age in days was plotted on the abscissa, whereas the logarithms of the total weight in g, and the logarithms of the DNA content in mg – to be read as number of diploid nuclei – were recorded on the ordinate. The data of ENESCO and LEBLOND's study lend themselves particularly well to a plot relating mitotic rate of tissues and organs to metabolically active body size. The Figure illustrates the relation between metabolic size ($kg^{3/4}$), on the abscissa, and mitotic rate (mg DNA), on the ordinate, for the whole rat, liver and heart at 7, 17, 50 and 90 days of age. The Figure illustrates relationships which in conventional plots go unnoticed.

It can be deduced from the Figure, for example, that in 90 days the rat bodies grew to full maturity, whereas the mitotic rate of their hearts and livers did not follow the same rate of change. Between the 17th and 50th day of age the mitotic rate of the liver, as compared to that of the heart, slows down considerably. This period, from weanling to the beginning of puberty, coincides with the appearance of tetraploid and the first octaploid nuclei in the liver[20]. The mitotic rate of liver cells thereafter, that is from the 50th to the 90th day of age, is marked by a



Relation between metabolic size ($kg^{3/4}$) and mitotic rate (mg DNA) for the whole rat, liver and heart in dependence of age (7, 17, 50 and 90 days of age). Re-plotted after ENESCO and LEBLOND[19].

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fast rate of change comparable only to that of the period preceding the 17th day of age. The 90th day approximates about the age of 20 years in man and the appearance in the liver of an octaploid DNA class[21].

The data and relationship for the two organs and the whole rat have been chosen only as an illustration. Similar plots for other organs and tissues for a variety of species may prove useful not only as basic information but may have practical implications. A few which come to mind immediately are the determination of optimal organ and tissue-specific radiosensitivity since the latter is known to vary with phases of the mitotic cycle[22]. A relation could also exist between animal mortality and the state of the animal's cellular populations[23]. Another use may be the calculation of optimal timing for the administration of certain classes of drugs and specifically of cytostatic compounds[24].

Zusammenfassung. Das Verhältnis zwischen Lebensdauer und stoffwechselbedingter Körpergrösse ($kg^{3/4}$) wird zur Analyse des Differenzialwachstums einzelner Gewebe und Organe herangezogen.

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PRO EXPERIMENTIS

Quantitative N-Terminal Amino Acid Analysis by Thin Layer Chromatography

The fluorodinitrobenzene reagent is widely used to detect the N-terminal amino acid in peptides and proteins[1]. A variety of column chromatographic procedures have been applied to identify and estimate the dinitrophenyl (DNP) derivatives[2–8], but these methods are laborious and time consuming or need expensive equipment. For these reasons the paper chromatographic technique described by LEVY[9] has gained in favour for quantitative determinations.

Recently, BRENNER et al.[10] applied thin layer chromatography to identify DNP-amino acids. This method has more sensitivity, gives better separation and consumes less time than paper chromatography.

Taking advantage of these convenient features we have developed a procedure for quantitative determination of N-terminal amino acid in proteins and peptides using chromatography on thin layers of silicagel.

Experimental. Since DNP derivatives are light sensitive, most operations must be performed in the dark.

0.05 to 1 μmole of the protein is dissolved in 5 ml of 0.1 *M*, pH 8.7, ammonium carbonate. If the analysis is carried out on small peptides, 1% aqueous trimethylamine[3] is used as solvent. Fluorodinitrobenzene (0.2 ml) is added and the mixture is vigorously stirred for 2 h at 40 °C. At the end of this period the excess of reagent is extracted with peroxide-free ether. To remove the remaining dinitrophenol, the extracted reaction mixture is dialyzed against distilled water and freeze-dried. This step is replaced by the sublimation technique of MILLS[6] when the method is applied to small peptides.

The purified DNP derivative is dissolved in glacial acetic acid avoiding any excess of solvent and the exact concentration of this solution is found by weight analysis[11]. The number of DNP groups present is estimated by ultraviolet absorption measurements. The following formulas are used:

$$C_1 = (1.67\ E_{290} - 0.51\ E_{370}) \times 10^{-4}$$

$$C_2 = (9.55\ E_{370} - 0.73\ E_{290}) \times 10^{-5}$$

where E_{290} and E_{370} are the absorbances measured at 290 and 370 mμ of the acetic acid solution and C_1 and C_2 are the molar concentrations of O-DNP-tyrosine and ε-NH_2-DNP-lysine respectively.

A known volume of the solution is hydrolysed with 5.7 *N* hydrochloric acid in a vacuum sealed ampoule at 100–110 °C. The acetic acid is eliminated in a flash evaporator before addition of hydrochloric acid to the ampoule. The destruction of the N-terminal amino acid derivative during the hydrolysis step is variable in each particular case[3]. The proper correction factors are estimated in parallel recovery experiments in which the unsubstituted protein is hydrolysed in the presence of a known amount of the adequate DNP-amino acid.

The N-terminal amino acid is extracted from the hydrolysate and isolated by thin layer chromatography according to BRENNER et al.[10]. The DNP-derivatives of aspartic and glutamic acids which run together in the original procedure, are resolved employing benzene:pyridine:glacial acetic acid (80:20:10) as second system of solvents.

The elution of the DNP amino acid from the chromatographic plate is performed as follows: the silica gel in the area of the DNP-amino acids spot is peeled off about 0.5 cm

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