regulation processes on the niveau of the forebrain may be influenced by hormones[3,6].

Zusammenfassung. Intramuskuläre Injektionen von Schafprolaktin in immobilisierte *Lepomis gibosus* und *Astronotus ocellatus* verändern die elektrische Aktivität von Vorderhirnneuronen; niedrige Dosen steigern, hohe hemmen sie. *Tilapia leucosticta und Carassius auratus gibelio* reagieren nur mit geringer kontinuierlicher Aktivierung.

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COGITATIONES

Hormones, Thymus and Lymphocyte Functions

The function of the thymus for the development of the immunological system is now well established. Recent work has proved that the thymus is under hormonal control[1-6] and evidence has been provided that the thymus may act as an endocrine gland and thereby influence other endocrine glands[7,8]. With but few exceptions the thymus-derived lymphocytes (T-lymphocytes) have thus far been investigated almost entirely with respect to their immunological functions. A major theme of this communication is that T-lymphocytes are the cellular end-product of hormones-thymus interrelation and have important functions quite apart from those familiar to the immunologist. This view is supported by numerous findings on the various wasting syndromes whose aetiopathogenesis and symptomatology serve as models for this thesis. In essence it is proposed that wasting syndromes express the intimate interrelation between the development of lymphatic tissue function and the endocrinological system and that the lymphocytes deriving from this interaction are *multifunctional*, since they can prevent the complex symptomatology of certain wasting syndromes (see Table I and II).

The term 'multifunctional lymphocyte' is used to refer to both classical immunological functions of T-lymphocytes and their participation in other less well defined homeostatic control mechanisms. It is entirely possible that these functions measurable by other than immunological parameters involve the same underlying mechanisms as the immunologic ones and may even be performed by the same lymphocytes[15]. In what way such 'nonimmunological' functions of lymphocytes should be exerted is not presently clear. One possibility is that lymphocytes have a 'trephocytic' function[10]. The existence of tissue specific factors produced by lymphoid cells and their significance in morphostasis has been proposed by Burwell[11]. Another consideration is that lymphokines may be set free upon stimulation of T-lymphocytes by certain tissue cells and in turn influence cells of various organs. It seems to us that a strictly immunological approach to the T-lymphocyte-derived mediators might prove to be a too limiting view of lymphocyte function.

Wasting syndromes as models for abnormal thymus-endocrine glands interrelation. In establishing the importance of the thymus much information has been obtained through study of the deficits produced by neonatal thymectomy in rodents, including the wasting syndrome, impaired cell-mediated immunity and to a lesser extent reduced humoral immune responses. The 'wasting' or 'runting' syndrome occurring in some strains of mice and in other species after neonatal thymectomy has been reviewed at length[16-31]. The prevailing view is that in neonatally thymectomized mice this syndrome is the result of immunological impairment. Studies performed in neonatally thymectomized mice born and bred in germ-

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free conditions have suggested that pathogen and non-pathogen viral and bacterial flora in these animals strongly influence the onset and course of the wasting syndrome[21–31].

Our approach to the aetiopathogenetic identification of wasting syndromes is based on recent evidence that hormones regulate and control the development and the differentiation of the immunolymphatic system[1–6, 32, 34–37] and that the thymus acts on other endocrine glands[7, 8, 32]. Accordingly it is proposed to elucidate and correlate the different and often apparently unrelated aetiopathogenetic causes of the wasting syndromes and view them in their broader biological context. BILLINGHAM[33], in his comprehensive analysis of the graft-versus-host reaction, has explored this problem most succinctly.

Table I. Symptomatology and pathological alterations in wasting syndromes

Pathological alterations	References
Inhibition and progressive decrease of body growth	3–5, 7, 16–19, 34–39, 41, 44, 47, 55–58, 64, 65
Thinness of the skin. Epidermal changes	3, 4, 7, 15, 18, 19, 33, 41, 47, 55–57, 64, 65
Lack of subcutaneous fat (*panniculus adiposus*)	3, 4, 7, 18, 19, 33, 34, 36, 38, 55–58, 64, 65
Length of ears and tail	4, 7, 36, 41
Microsplancnia and microsomia	4, 7, 36, 38, 39, 41, 47
Ruffled, juvenile-type hair	3, 4, 16–19, 34, 36, 38–40, 55–57
Reduction in number and size of hepatic cells	38
Foci of necrosis in the liver and/or spleen	18, 19, 25, 33, 38, 39, 58
Hemorrhagic diarrhea	16–19, 33, 34, 38, 39, 55–57
Atrophy of the thymus (in nonthymectomized animals) and of peripheral lymphoid organs. Lymphopenia in peripheral blood	3, 4, 7, 16–19, 33, 34–36, 38, 39, 55, 56–58, 64, 65
Osseal alterations	3, 4, 19, 33, 36, 41, 48–51, 57
Hunched posture or kyphosis (deriving from incomplete development or malformation of the vertebrae)	3, 4, 7, 34, 37, 41, 57
Degranulation of acidophilic cells in the hypophysis	5, 7, 32, 34, 66
Bone marrow atrophy and focal necrosis	3, 4, 7, 33, 39
Microcytic anaemia	4, 33, 39, 57
Different sex incidence	42, 43, 57
Sterility of males or of females, ovarian dysgenesia	4, 38, 41, 44, 64
Atrophy of thyroid gland	4, 7, 38, 41, 62, 63
Alterations of adrenal cortex	7, 8
Lack of appearance of secondary sex characters in males, submaxillary gland and kidney fail to develop, spermatogenesis is incomplete	4, 38, 41, 64

Table II. Types of wasting syndromes

	References
a) *Wasting syndromes with no evident direct participation of endocrine factors*	
Secondary disease after radiation	45
Salmonella typhimurium infection	46
Vaccine-induced wasting syndrome	47
Virus-induced wasting syndrome	52
Wasting disease induced by heterologous anti-thymocyte serum	53, 54
Homologous disease or runting disease	33
b) *Wasting syndromes with evident direct participation of endocrine factors*	
Hydrocortisone-induced wasting disease	3, 55, 56
Testosterone-induced wasting disease	57
Wasting disease induced by estrogens	58
c) *Wasting syndromes with possible participation of endocrine factors*	
Wasting disease induced by heterologous anti-lymphocyte serum	59
Wasting disease after neonatal thymectomy	16–19
Wasting of thymusless 'nude' mice	7, 8, 64, 65
Ageing	15, 60, 61, 68
Malnutrition and stress	51, 57
d) *Wasting syndromes induced by interruption of endocrine-thymus relationship during ontogenic formation of the immunolymphatic system*	
Age-dependent wasting disease after injection of heterologous anti-hypophysis serum in young adult mice	1, 2, 34, 37
Age-dependent wasting disease after injection of anti-somatotropic hormone serum into young adult mice	1, 35, 37, 67
Hypopituitary dwarfism or post-hypophysectomy syndrome	4, 15, 36, 37, 41
Experimental diabetes (alloxan induced or following pancreatectomy)	63
Age-dependent wasting disease following abolition or inhibition of thyroid function in newborn or young adult mice (treatment with ^{131}I, propyl-thio-uracil or anti-thyrotropic hormone serum)	63

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Our approach to classify and understand the wasting syndromes involves several assumptions: 1. the thymus is involved in all kinds of wasting disease, either primarily or at later stages of their development; 2. the variously induced endocrine disturbances lead to alteration in thymus activity and to a deficit in the formation of hormone-dependent thymus-derived lymphocytes; 3. disturbance of the endocrine function of the thymus during early ontogeny leads to a disturbed function of other endocrine glands resulting in a quantitative and qualitative deficit in the mature, multifunctional thymus-derived lymphocytes with consequent expression as wasting syndromes.

Symptomatology and pathological alterations in wasting syndromes. A number of derangements in wasting syndromes which are either naturally occurring or can even be experimentally induced by various methods have been recorded. Some of the most frequently observed alterations are listed in Table I.

It is noteworthy that irrespective of the mode of induction, wasting syndromes are characterized by a remarkable similarity in their overall manifestations. Furthermore, it seems to us hardly coincidental that most of these very same stigmata are also seen in the hypopituitary dwarf mouse[4,15,36,37], in thymusless nude mice[7,8] and in the various stages of the post-hypophysectomy syndrome in mammals[41].

Some of these effects such as degranulation of acidophilic cells in the anterior pituitary gland, lymphatic aplasia a.o. can also be found in apparently normal animals[5,32,34,66]; this could be viewed as presaging eventual emergence of the full syndrome.

Types of wasting syndromes. Among the different wasting syndromes are a) those which apparently do not directly involve the endocrine system, b) those which are definitely produced by exposure to various hormones, c) those where participation of endocrine factors is suspected, d) those induced by interruption of the thymus-endocrine glands relation.

The common feature of the wasting syndromes listed in Table II is an involvment of the thymus and thymus-dependent lymphoid tissues, a point that is featured in our thesis that the ontogenic development of thymus and the endocrine system are interdependent.

Non-immunological implications of wasting and runting in mice. From Table I and II there is thus evidence that many symptoms, alterations of functions and pathological changes in 'runting' or 'wasting' animals are not directly dependent on impaired immunological function. It is also apparent that many of these symptoms are manifestations of endocrine alterations in the animals before and after the onset of the wasting syndrome. Indeed there is hardly any facet of wasting in rodents which is not equally interpretable as an alteration in endocrine functions[4,7,36,37,40,41,67].

Accordingly it seems to us curious that infectious processes have been given much more prominence than endocrine disturbances as the primary cause of some wasting syndromes. That bacteria and viruses participate in inducing the onset, increasing the incidence, speeding the course and rendering more manifest the typical symptoms of some wasting syndromes is not in doubt. However, in our view, neither infectious processes nor immunodeficiency states or their combination need be the primary cause of wasting syndromes. The apparently logical dependence of wasting syndromes on immunodeficiency is based on the prevention of the wasting syndrome of neonatally thymectomized mice under germfree condition[21,31] and on its prevention by lymphocytes under conventional conditions. This is in our view an oversimplification based on consideration of one parameter only i.e. the classical immune function of the lymphocyte. To prove this one should be able to show that prevention of these syndromes by treatment with peripheral lymphocytes is dependent only on their function as antibody producers or as effector cells in cell-mediated immunity. Consequently the concept that wasting syndromes derive originally from spreading of bacterial or viral infectious processes in animals with a deficient immunological resistance only, is considered by us as too limiting and narrow an interpretation as would be the suggestion that the wasting syndrome is provoked by bacterial toxins[29,30]. Bacteria and viruses would however be capable of *amplifying* or even being a determinant in revealing a *pre-existing* deficiency in the lymphoid tissue. Although impairment of immunological functions, such as deficiency in antibody production to certain antigens or impaired cellular immunity, has been established in some of the experimental systems used to induce a wasting syndrome, the extreme variability of the immune capacity among the different species and even the strain differences in the same species suggest that the immunological deficiency in the different experimental conditions for the development of a wasting syndrome is not the major determining factor for its induction. Therefore the prevention of death of neonatally thymectomized mice raised in germfree condi-

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tions does not suffice to support the concept of presence of immunodeficiency only in the thymectomized mice. We suggest that the cause of wasting is the variously induced disfunction of the thymus in a critical period of its hormone-dependent development or when it exerts its endocrine function on other endocrine glands, which leads to immunological, endocrine and other deficits (see Table I and II).

Do wasting diseases involve alteration of the feed-back hormonal control on the thymus? The involution of thymus and thymus-dependent lymphoid tissue is a characteristic of wasting syndromes. Whatever the cause for a disturbance of thymus function, be it removal of the neonatal thymus or its destruction, involution or atrophy at a certain stage of its growth and differentiation, such alteration of the thymus either precedes or accompanies the development of the wasting disease. Accordingly it is proposed that a basic prerequisite for appearance of a wasting syndrome is an attack against thymus integrity, growth, differentiation and function during its hormone-dependent development. Correspondingly the difference in the occurrence of the wasting syndromes in the different species would thus be dependent on the clearly established distinctive species-specific character of ontogenic development of the thymus. It would of course be important to know whether the appearance or course of the syndrome is directly dependent on the loss of thymus function. It is, however, difficult to envisage that all the various symptoms of the wasting syndromes should be solely dependent on the abolished thymus function. Rather it seems to us likely that the destruction of the thymus function has itself provoked a wider and more complex alteration due to a disruption of the thymus-endocrine glands relationship in ontogeny. Among the ever widening repercussions of these events there ensues a lack of formation of mature multifunctional T-lymphocytes deriving from the missing developmental effects of hormones on thymocytes. Experimental evidence for the above propositions derives from the demonstration that many of the wasting or runting syndromes, enumerated in Table II, can easily be produced by treatment with some hormones or by interfering with hormonal activity provided the hormone-dependent attack on the thymus is exerted with critical modality of time and dose[1,2,34,37,53,55,57,58,66,67]. Our findings on the effect of neonatal thymectomy on the hypophysis in mice[34,66] confirmed by electron microscopy studies in neonatally thymectomized conventional or germ-free mice[5,32], those on the effect of anti-pituitary serum and anti-somatotropic hormone serum in mice[34,35,67] and of somatotropic hormone on reconstitution of dwarf mice[4,36] are judged to be experimental evidence for the relationship between neonatal thymus and hormones[1-6,34-37,66,67]. Also our recent findings on adrenal and thyroid alterations in athymic nude mice[7,8] support this contention.

Hormones have so far been mainly considered as non-specific factors in immune processes despite the knowledge that they are among the most ancient and complex polypeptide molecules appearing in phylogeny. It should not be surprising that hormones have in evolution acquired the function of controlling such a sophisticated system as the lymphoid tissue. It is probable that function and interdependence of endocrine and lymphoid systems developed in a parallel fashion in phylogeny. Thus some endocrinological alterations, as for example the precocious degranulation of acidophilic cells in the hypophysis of neonatally thymectomized wasting or nonwasting, conventional or germ-free mice, the changes in the adrenal cortex of athymic nude mice and other symptoms and alterations might express this link (see Table I).

Are there 'nonimmunological' functions of lymphocytes? The mixed heterogeneous cell population of the thymus might control through its final product, the hormonally conditioned mature T-lymphocyte, a number of other functions which have little or nothing to do directly with classical immunological reactions. It has been shown[15,69-73] that retarded or impaired skin and hair growth and differentiation, alteration in bone growth, disfunction of endocrine glands, delay or lack of sexual maturation, deficiency of exocrine gland secretion in neonatally thymectomized animals can be prevented by supply of lymphocytes. Thus experimental evidence exists of the capacity of these cells to influence physiological processes of a nonimmunological character. Whether this involves a direct participation of lymphocytes or whether it is due to their indirect influence through the endocrine system is unknown[7,8,15]. It therefore seems evident that the immunological failure parallels failure of other functions in the wasting condition. On this basis it seems to us that the immunological concept of the thymus as representing *only* a source of cells of significance for antibody production and cell-mediated immunity is too narrow and may well be misleading. However, in mammals a morphological or functional differentiation of lymphocytes or thymus-derived cells involved in other functions is not yet possible. The death of the runting mouse in homologous disease, of the neonatally thymectomized mouse or the hypopituitary thymus-deficient dwarf mouse and of the thymusless nude mouse may in the final analysis be caused by infection. However, nobody has proved that in reconstituting neonatally thymectomized mice with lymphoid cells or preventing runting in homologous disease with syngeneic adult lymphocytes or producing immune recovery and prolonging life of dwarf mice with adult lymphocytes[4,15], one is providing *only* precursors of immunocompetent cells or mature immunocompetent cells with classical immune function. The more or less marked immunological deficiency occurring in the wasting syndromes we believe to be only *one* expression of the impairment of functions provoked by the attack on the thymus during its formation in the period of its maximal hormone-dependent cellular activity and while acting itself as an endocrine gland.

How can a nonimmunological function of lymphocytes be visualized? Several concepts have been proposed[9-15]. The recent discoveries of mediators of cell-mediated immunity, produced by stimulated T-lymphocytes and their varied action on different cell types would of itself make for a new more generalized interpretation. Accordingly we propose that tissue cells of many organs could interact with T-lymphocytes which are thereby stimulated to produce and release mediators which stimulate tissue cells in their immediate environment to mature and divide.

Implications for senescence. Several immunological theories on the ageing process have been proposed and have recently been summarized by WALFORD[60] and BURNET[68]. Immunological[60,68] and endocrinological aspects[74] have been considered separately in connection with ageing, whereas the derivation of a multifunctional lymphocyte from the action of hormones on thymus and

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possibly on thymus-derived lymphoid cells has not been reported in the literature. We concur with the aforementioned view that senescence should be regarded as a kind of protracted wasting disease but we assume that in many wasting syndromes the primary cause for the progressive diminution of the available pool of hormone-dependent, multifunctional thymus-derived lymphocytes lies in the endocrine system. Examples in support of this view are the different wasting or runting syndromes, the hormonally deficient short-lived hypopituitary dwarf mice whose life can be considerably prolonged by either hormone treatment or by injection of lymph node lymphocytes[15], and the short-lived hormone-deficient thymusless 'nude' mice[7,8]. The progressive deterioriation of this hormone-lymphoid cells relationship seems eventually to be facilitated or accelerated by many secondary causes such as infection, stress, trauma and malnutrition.

Many of these accelerating causes of senescence might derive from premature destruction of hormone-sensitive thymocytes or thymus-derived lymphocytes by hormones such as gonadotrophins and adrenal or gonadal steroids. In fact, in many conditions of stress, both emotional and muscular, the levels of STH, steroids and other pituitary trophins vary greatly[75–81]. Therefore in seeking to unravel the complex process of ageing the hormonal factors regulating cells turnover in the thymus and in the thymus-derived tissues deserve special attention.

Zusammenfassung. Es wird die Hypothese vorgeschlagen, dass Thymus-Lymphozyten (T-Zellen) neben ihren klassischen immunologischen Funktionen wichtige multifunktionelle homöostatische Kontrollfunktionen ausüben. Störungen in der Beziehung zwischen dem endokrinen System und Thymus führen zu Ausfallerscheinungen und Altern.

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PRO LABORATORIO

Automated Specimen Processing for Electron Microscopy: A New Apparatus

While automated specimen processing is available for paraffin embedding in light microscopy, plastic embedding for electron microscopy is still carried out manually in spite of the fact that electron microscopy is being increasingly applied to routine (e.g. diagnostic) work and that plastic embedding is increasingly used also for light microscopy employing 0.1–1 micron-thick sections. Among the problems in automating plastic embedding are: the great variety of procedures, the small size of the specimens, the requirements for cleanliness, precision and reproducibility of the procedure, the incompatibility of some chemicals with machine parts, the viscosity and pre-polymerization of the embedding resins, etc.

We now present a simple and compact apparatus which automatically dehydrates and impregnates up to 24 specimens simultaneously with up to 29 sequential changes of 10 different fluids.

Description. The main components of the apparatus (Figure 1) are: a removable support with reservoir flasks, silastic tubes closed by electromagnetically driven guillotine-type valves, a 100 ml polypropylene trough, a moving holder with 24 disposable specimen baskets (Figure 2), a 5 l container for disposed liquids, a programme-selecting panel with indication lights (Figure 3), and an electric unit with 4 switches: 'Main', 'Start', 'Step', and 'Reset'. The size of the apparatus (without flask support) is $31 \times 35 \times 40$ cm, and its weight is 20 kg.

Mode of operation. When 'Main' and 'Start' are pushed, the apparatus fills 100 ml fluid from any pre-selected flask into the trough, moves the specimen baskets up and down for a pre-selected time period, then stops the movement, empties the trough and switches over to the next step, which begins with refilling the trough from the same or another flask. Up to 29 such steps can be programmed by placing 2 pins for each step into the programme-selecting panel: 1 to determine the flask (A-K), and 1 to select the time period (0, 1, 2, 3, 5, 10, 15, 30, 45 or 60 min per step). If a time position is left without a pin, the apparatus operates on this position until the 'Step' switch is pushed. This enables the apparatus to operate on one position for a longer period without emptying the trough, e.g. overnight. Thereby, any evaporation of fluid is compensated by automatic refilling from the corresponding flask. In addition, the 'Step' switch can be pushed at any moment in order to immediately empty the trough and pass to the next step independently of the actual time setting, whereas with the 'Reset' switch the programme can be interrupted at any moment by proceeding directly to the initial zero position.

Special features. Self-cleaning of the apparatus is achieved after the programme is finished and the specimens are taken out by simply adding some more steps, whereby a resin solvent or another cleaner is to be filled into some of the flasks. Any of the common dehydrating fluids, resin solvents and resins which are compatible with silicone, teflon, polypropylene and stainless steel can be used, e.g. water, buffers, aldehydes, ethanol, acetone, propylene oxide, Epon, etc. The viscosity of the fluids may be as high as that of unpolymerized Epon resin and the viscous fluids can be stirred by magnetic stirrers which are placed under 2 of the flasks. Fluids are used only once and the specimen baskets are disposable.