thyroidism, fixed in cold Bouin for light microscopy and in Palade's fixative buffered with sucrose at pH 7.4, embedding in Vestopal-W; the ultra-thin sections were cut by LKB-ultrotome, mounted on grids and stained by lead citrate before examination[7], under JEM-5Y.

Examination by light microscope points to a compact, cordonal structure in control dogs, separated by connective tissue and blood capillaries. In hypoparathyroid dogs, we observed an intense atrophy, disorganization of cellular pattern with degenerative cell lesions, fibro-lympho-plasmocytic infiltrations and advanced sclerosis.

Electron microscopy showed in parathyroid of control dogs many oval cells separated by fine intercellular spaces, oval or elongated nuclei, enveloped by a double nuclear membrane; unexpended endoplasmic reticulum as small empty vesicles and 2 kinds of opaque secretory granules – first kind of large, medium dense electron granules, oval and bounded by a single membrane ranging in size from 0.5–0.8 μ; the second is smaller, measuring 100–200 nm, oval and contains a very dense electron substance; mitochondria are elongated with cristae; many free-ribosomes in matrix of cytoplasm. At the basal zone, there are some parathyroid cells and endothelial cells, with evident basement membrane separating them from a capillary lumen and red blood cells.

In the parathyroid gland of dogs with hypoparathyroidism, we noted severe ultrastructural changes, which consist of atrophy of endoplasmic reticulum, reduction of number and size of secretory granules. Nuclei are elongated enveloped by a very irregular outline of nuclear membrane, giving a cog-like pattern. Mitochondria are swollen and vacuolated with disrupted cristae and irregular outline. Rarely, we saw at the basal zone some electron medium dense granules, their lumen being half empty and moving towards the periendothelial space. Frequently, we noted large zones of sclerosis formed by many fibrocytes and connective fibres with typical periodicity and encircling the dislocated epithelial cells.

From this study it appears that, in experimental hypoparathyroidism in dogs, the ultrastructural and structural pattern is very damaged. Thus, in most specimens the atrophy of endoplasmic reticulum, tumefaction and vacuolation of mitochondria, reduction of number and size of secretory granules, are the prominent ultrastructural and structural features. All these fine structural changes may explain the decrease in the parathormone (PTH) secretion rate, and consecutively the upset in phosphocalcium metabolism.

However, it is difficult at present to conclude about the mode of secretion and release of PTH, and thus whether PTH can be identified by electron microscopy; it is possible, as in other endocrine glands (thyroid, adrenalcortex), that these granules represent the first step of synthesis of PTH, or represent some form of stored intracellular product; the increase of these granules in the vicinity of Golgi zone in hyperactive parathyroids suggest this. Some authors suggest that first type of granules with medium electron density represent the first step in synthesis of PTH[8], so-called prosecretory granules and the dense granules, the final stage, of storage of PTH, being secretory granules[2]. Both kinds of granules are very much decreased in hypoparathyroid dogs.

These findings may represent a new contribution to the experimental cytophysiology of the parathyroid gland.

Résumé. Les modifications ultrastructurales de la glande parathyroïde sont étudiées ici chez les chiens témoins et les chiens avec hypoparathyroïdisme produit par isoimmunisation. On observe une réduction du reticulum endoplasmique des granules sécrétoires ainsi qu'une tuméfaction et vacuolisation des mitochondries; les noyaux ont un aspect dentelé chez les chiens hypoparathyroïdiens. Ces données peuvent apporter une contribution à l'étude de la cytophysiologie de la glande parathyroïde.

A. Lupulescu[9], A. Petrovici, A. Pop and C. Heitmanek

Institute of Endocrinology and 'Dr. I. Cantacuzino', Bucharest (Romania), 8 May 1967.

[7] E. Reynolds, J. Cell Biol. *17*, 208 (1963).

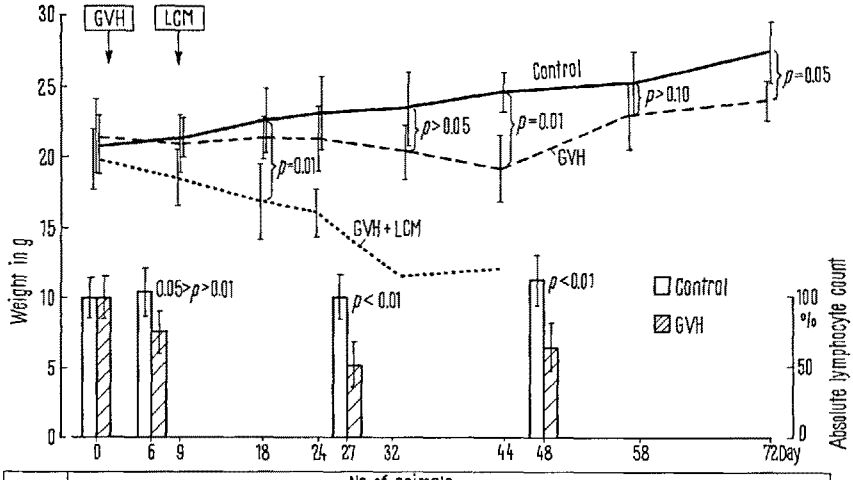
[8] R. Davis and A. Enders, in *The Parathyroids* (Eds R. O. Greep and R. V. Talmage, C. H. Thomas, Springfield 1961), p. 76.

[9] Present address: Università di Roma, Istituto di Patologia Medica Roma (Italy).

Interaction of Graft-versus-Host Reaction and Lymphocytic Choriomeningitis Infection in Mice

Characteristic features of graft-versus-host (GVH) disease are lymphoid hypoplasia and impaired immunological reactivity with a low level of circulating antibodies and immunoglobulins[1–7]. In such animals, susceptibility to infections and toxic substances seems to be increased. Even the 'runting' or 'wasting', developing in the course of GVH reaction, is supposed to be the result of toxic influences and/or infection[6–8]. It is also known that some viruses, especially in immunologically incompetent animals, can lead to a state of runting[9,10]. On the other hand, it was shown that occurrence of fatal meningitis in mice infected intracerebrally with an appropriate dose of the virus of lymphocytic choriomeningitis (LCM) can be greatly reduced by thymectomy or by other treatment resulting in lymphoid depletion and immunosuppression[11–16]. Further, there exists some parallelism between thymectomy and the GVH reaction[17–19]. On the basis of the above data, a study of the interaction of GVH reaction and LCM infection seemed to be of interest.

GVH reaction was produced in 6- to 8-week-old (C_{57}Bl × CBA) F_1 hybrid mice of both sexes. The animals were injected i.v. with 50×10^6 spleen cells from adult C_{57}Bl donors, matched according to sex. Controls received (C_{57}Bl × CBA) F_1 hybrid cells, using the same technical procedure. The pre-titrated 300 LD_{50} dose of LCM virus was inoculated intracerebrally on the 7th day following spleen cell transfer. The weight, mortality rate, signs of runting, and neurological symptoms respectively were regularly recorded. In mice not infected with LCM virus, absolute lymphocyte counts were done at intervals.



	No. of animals							
Control	16	16	10	10	10	8	7	7
GVH	16	15	14	13	10	7	4	4
GVH+LCM	32	31	15	5	2	1	0	0

Fig. 1

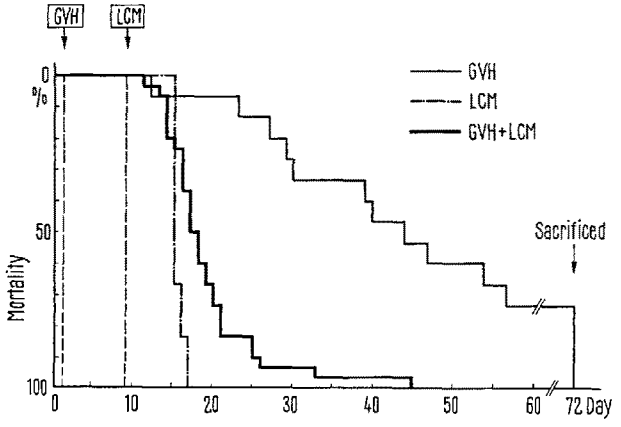


Fig. 2

Some mice of the GVH-LCM group were examined for the presence of LCM virus. Grouping and numbers of mice are shown in Figure 1.

Control mice gained weight throughout the experiment and the 3 animals lost in this group died accidentally from technical causes. On the 9th day of the experiment, 6 of the control animals were infected with LCM virus. These mice exhibited the typical neurological picture of choriomeningitis and all died between the 15th and 18th day, i.e. within 8 days following LCM infection. The weight curve of mice receiving parental cells only declined slowly. Concomitantly, these animals showed signs of runting of varying intensity. Appearance of mortality was gradual, starting on the 9th day after cell transfer; at the end of the experiment, 25% of the animals of this group were alive and showed, from the 44th day, some gain in weight. The establishment of the GVH reaction in this group was also confirmed by the significant decrease of the absolute number of circulating lymphocytes (Figures 1 and 2).

Mice of the GVH-LCM group showed rapid weight loss. If compared with the LCM-infected controls, the acute mortality was decreased in this group (Figures 1 and 2). Neurological signs were observed only in some of the animals succumbing between the 4th and 11th day following LCM infection, i.e. in the relatively early period of the GVH reaction; mice surviving this period showed no neurological symptoms (Table). However, in this group signs of runting appeared earlier and were more pronounced than in animals with GVH disease alone, and, in function of time, the overall mortality rate was between that of control mice infected with LCM and that of mice of the GVH group (Figure 2). In 5 animals surviving LCM infection 16, 17, 24, and 37 days respectively, the virus-carrier state could be demonstrated.

[1] J. G. HOWARD and M. F. A. WOODRUFF, Proc. R. Soc. B. *154*, 532 (1961).
[2] H. OLINER, R. SCHWARTZ and W. DAMESHEK, Blood *17*, 20 (1961).
[3] M. SIMONSEN, Progr. Allergy *6*, 349 (1962).
[4] R. M. BLAESE, C. MARTINEZ and R. A. GOOD, J. exp. Med. *119*, 211 (1964).
[5] M. KOLTAY, R. G. KINSKY and B. G. ARNASON, Nature *205*, 509 (1965).
[6] M. KOLTAY, R. G. KINSKY, B. G. ARNASON and J. B. SHAFFNER, Immunology *9*, 581 (1965).
[7] R. MCBRIDE, Cancer Res. *26*, 1135 (1966).
[8] J. G. HOWARD, Nature *190*, 1122 (1961).
[9] J. HOTCHIN and H. WEIGAND, J. Immun. *86*, 392 (1961).
[10] N. F. STANLEY, Med. J. Aust. *2*, 815 (1961).
[11] R. H. LEVEY, N. TRAININ, L. LAW, P. H. BLACK and W. P. ROWE, Science *142*, 483 (1963).
[12] W. P. ROWE, P. H. BLACK and R. H. LEVEY, Proc. Soc. exp. Biol. Med. *114*, 248 (1963).
[13] J. EAST, D. M. PARROTT and J. SEAMER, Virology *22*, 160 (1964).
[14] P. FÖLDES, I. SZERI, ZS. BÁNOS, P. ANDERLIK and M. BALÁZS, Acta microbiol. hung. *11*, 277 (1964).
[15] J. HOTCHIN and E. SIKORA, Nature *202*, 214 (1964).
[16] I. SZERI, ZS. BÁNOS, P. ANDERLIK, M. BALÁZS and P. FÖLDES, Acta microbiol. hung. *13*, 255 (1966).
[17] J. F. A. P. MILLER, A. H. MARSHALL and R. G. WHITE, Adv. Immun. *2*, 111 (1962).
[18] K. R. MACINTIRE, S. SELL and J. F. A. P. MILLER, Nature *204*, 151 (1964).
[19] D. M. PARROTT and J. EAST, *The Thymus in Immunobiology* (Hoeber Medical Division, Harper and Row, New York 1964), p. 523.

Thus, our preliminary observations show that: (1) early mortality and neurological manifestations of LCM virus infection are reduced in mice undergoing GVH reaction; and (2) the characteristic runting syndrome of GVH mice is precipitated and aggravated by LCM infection.

The data presented substantiate our earlier observations on decreased immunological reactivity of GVH mice[5,6]. Further, it seems that development of active neurological symptoms and early mortality in LCM-infected mice are closely correlated to availability of active lymphocytes. In our series, no such manifestations were noted on the height of GVH reaction when the lymphocyte count was the lowest. This is in accordance with the recently published observation of others that early mortality of LCM infection can be prevented by treatment with anti-lymphocyte serum[20]. The fact that in our present study the LCM infection enhanced and aggravated the runting syndrome established by the GVH reaction, is also in compliance with our earlier experiments made on neonatally thymectomized mice. Here, too, the runting syndrome elicited by thymectomy was considerably aggravated by LCM infection[16].

Our observations favour the hypothesis that runting or wasting is a non-specific syndrome, which may be caused by a variety of factors, among them viruses, in organisms with impaired immunological competence.

Mortality and incidence of neurological symptoms in mice undergoing graft-versus-host reaction and infected with virus of lymphocytic choriomeningitis

Days after LCM infection	5	6	7	8	9	10	11	12	16	17	24	37
No. of mice succumbed	4	1	4	4	3	2	2	3	2	1	1	1
No. of mice showing neurological symptoms	2	0	0	3	3	1	1	0	0	0	0	0

Résumé. Chez des souris hybrides F_1 adultes, subissant la maladie homologue produite par l'injection de cellules parentales, la mortalité précoce et les manifestations neurologiques d'une infection due au virus de la choriomeningite lymphocytique étaient réduits. D'autre part, le développement des symptomes caractéristiques du «runting» apparaît comme accéleré et aggravé par l'infection produit par ce virus.

M. Koltay, I. Virág, Zs. Bános, P. Anderlik and I. Szeri

Pediatric Clinic, University Medical School, Szeged and Institute of Microbiology, University Medical School, Budapest (Hungary), 31 July 1967.

[20] A. W. Gledhill, Nature *214*, 178 (1967).

A Study on the Immunological Properties of Human Uterine and Placental Contractile Protein by Immuneadherence[1]

In 1965, King and Gröschel-Stewart isolated and characterized a contractile protein from human term placenta (PCP)[2] that exhibited properties similar to both human skeletal actomyosin (SAM) and the uterine contractile protein (UCP) described by Needham[3]. Several reports on the immunological behaviour of contractile proteins from various sources were published since[4–6]; and these studies suggested antigenic differences between skeletal, cardiac and smooth muscle contractile protein. Antiserum prepared by us against human UCP did not produce a reaction with SAM; however, precipitation did occur with both UCP and PCP under the indication of identity bands (Figure). In agreement with the previous investigators, we obtained multiple band formation. On the other hand, antiserum against highly purified SAM did not react with UCP; although a single precipitation band was obtained with the homologous antigen[7]. Attempts to establish a quantitative relationship between the above mentioned antigens and the antiserum by precipitation reaction[8] were inconsistent and therefore unsatisfactory; apparently not only due to the heterogeneity of the antigens used, but also due to the high ionic strength required for antigen solubility, which reduces precipitation rate of antigen-antibody complexes[9]. We therefore felt that the principle of immuneadherence (IA), extensively studied by Nelson[10,11], would offer a method suitable for demonstrating large molecular weight antigens, and a possibility of semiquantitative determination of the antigenicity of the proteins studied. IA has been described as a specific immunological reaction wherein particulate and soluble antigens sensitized with antibody and complement become attached to the surface of human erythrocytes, due to the presence of an IA receptor on the membrane of the erythrocyte. Utilizing this method, we were able to establish the immunological relationship between UCP and PCP.

The antigens (AG), actomyosin from surgical specimens of human striated muscle SAM, UCP and PCP were isolated as described elsewhere[2,12]. Antiserum (AS) was obtained from rabbits immunized according to Fink[4]. The animals were bled by cardiac puncture. The antiserum was absorbed 4 times with the same type human

[1] Herrn Prof. Dr. Dr. W. Gröschel zum 60. Geburtstag gewidmet.
[2] Th. M. King and U. Gröschel-Stewart, Am. J. Obstet. Gynec. *93*, 253 (1965).
[3] D. M. Needham and J. M. Williams, Biochem. J. *89*, 552 (1963).
[4] H. Fink, Biochim. biophys. Acta *111*, 208 (1965).
[5] A. C. Fox and M. D. Klein, Biochim. biophys. Acta *127*, 232 (1966).
[6] D. Ricken, Dt. med. Wschr. *39*, 1717 (1965).
[7] U. Gröschel-Stewart, unpublished results.
[8] I. G. S. Furminger, Biochim. biophys. Acta *90*, 521 (1964).
[9] C. F. Kabat, E. A. and M. M. Mayer, Experimental Immunochemistry 2nd edn (C. C. Thomas, Springfield, Ill. 1964).
[10] D. S. Nelson and R. A. Nelson, Yale J. Biol. Med. *31*, 185 (1959).
[11] R. A. Nelson, Science *118*, 733 (1956).
[12] U. Gröschel-Stewart and F. Turba, Biochem. Z. *337*, 104 (1963).