Wasting syndrome in neonatal mice after administration of salivary gland homogenate

K. Tuch and Th. Matthiesen[1]

Department of Experimental Pathology, Chemie Grünenthal GmbH, D-5190 Stolberg (Federal Republic of Germany, BRD), 15 October 1976

Summary. A wasting syndrome, similar to that occurring after cortisol treatment, was induced in neonatal mice by means of the daily i.p. administration of salivary gland homogenate: 24 h after a single injection of the homogenate, profuse cell necrosis was observed in the thymic cortex, 48 h later the cortex was devoid of lymphocytes. It is hypothesized that the submandibular glands of mice contain substances which are capable of inducing a cortisol-like effect.

The submandibular gland of rodents is said to possess exocrine and endocrine properties, and there have been reports in the literature on a number of differently acting factors in the salivary glands[2-6]. One factor which is of particular interest is the inhibition of the lymphatic tissues, which produces thymic atrophy[7,8]. Mechanical or hormonal thymectomy in neonatal mice induces the wasting syndrome[9,10]. We therefore looked into the question of whether salivary gland homogenate can cause a similar disorder in the development of neonatal mice.

Material and methods. One half of a litter of neonatal NMRI mice received salivary gland homogenate i.p. (SH; group 1) and the other half, liver homogenate (LH; group 2). One half of another litter were given hydrocortisone acetate (HCA; group 3) s.c. and the other half were treated with the solvent (S; group 4), in both groups on the first and third day post partum (p.p.). The SH group was sacrificed on the 18th day p.p. and the HCA group on the 11th day p.p.

Preparation of the homogenate: the salivary glands (submandibular) of male NMRI mice weighing about 18 g were homogenized together with 5 ml 0.9% sodium chloride per 1 g tissue and centrifuged at 12,000 × g for 10 min[8]. The clear supernatant was diluted 1:10 in a 0.9% sodium chloride solution and 0.05 ml i.p. was given daily to each animal. Liver homogenate was prepared in the same way. Weight determinations: body weight; absolute and relative weight of the thymus, spleen, liver, kidneys and salivary glands. Histology: the organs were fixed in Schaffer's mixture, embedded in methacrylate[11], sliced and stained in haemalum eosin.

Results. 4–5 days after the beginning of the treatment, the development of the animals in group 1 was retarded compared with the other animals of the same litter. The incisors appeared on the 8th day and the eyes opened on the 12th day p.p., both events occurring 2 days earlier than in the controls. There was a delay in the initial growth of the fur. The symptoms in the HCA group were identical. Group 4 developed normally. The body weights of groups 1 and 3 were lower than those of the controls. The absolute and relative weight of the thymus and spleen was drastically reduced (figures 1 and 2). Histological examination revealed thymic atrophy after SH treatment. The cortex was practically devoid of lymphocytes and the structure of the reticular cells stood out as a result. The medulla, on the other hand, contained a large number of lymphocytes. It was possible to plot the cell depopulation mechanism in the cortex of neonatal mice after a single administration of salivary gland homogenate. 4 h after the injection, only isolated cells had been destroyed; 24 h later there was profuse karyorrhexis in the cortex (figure 3). 48 h later most of the cell debris was phagocytosed and the cortex contained only few lymphocytes, whereas there were many in the medulla (figure 4).

Discussion. The changes in neonatal mice caused by salivary gland homogenate are similar to the wasting syndrome induced by cortisol acetate. Consequently it can be concluded that the salivary glands contain substances which have a cortisol-like effect. Histological examination of the thymus showed that after the single administration of salivary gland homogenate many of the lymphocytes in the thymic cortex are destroyed and phagocytosed within 24 h. After 48 h the cortex no longer contains any

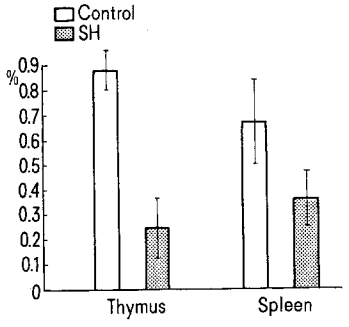

Fig. 1. Relative organ weight of the thymus and spleen after the administration of salivary gland homogenate (SH). Control = liver homogenate.

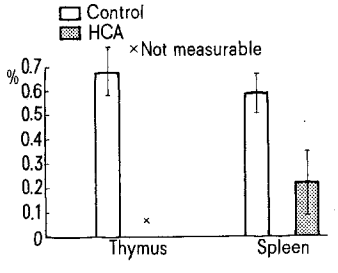

Fig. 2. Relative organ weight of the thymus and spleen after the administration of hydrocortisone acetate (HCA). Control = solvent.

1 We should like to thank Miss Angela Würfler for her careful technical assistance.

2 R. Levi-Montalcini and S. Cohen, Ann. N.Y. Acad. Sci. *85*, 324 (1960).
3 S. Cohen, J. biol. Chem. *237*, 1555 (1962).
4 P. U. Angeletti, M. L. Salvi, F. Capani and L. Frati, Biochim. biophys. Acta *111*, 344 (1965).
5 K. Hoshino and C. D. Lin, Cancer Res. *28*, 2556 (1968).
6 K. Hoshino and C. D. Lin, Can. J. Physiol. Pharmac. *47*, 329 (1969).
7 T. Takeda, Y. Yamasaki, H. Yamabe, Y. Suzuki, H. Haebara, T. Irino and A. Grollmann, Proc. Soc. exp. Biol. Med. *126*, 212 (1967).
8 T. Takeda and A. Grollmann, Am. J. Physiol. *215*, 1337 (1968).
9 D. M. V. Parrot, M. A. B. de Sousa and J. East, J. exp. Med. *123*, 191 (1965).
10 J. W. Jutila, J. Immun. *102*, 963 (1969).
11 H. Westen, Proc. Eur. Soc. Study Drug Toxicity *15*, 313 (1973).

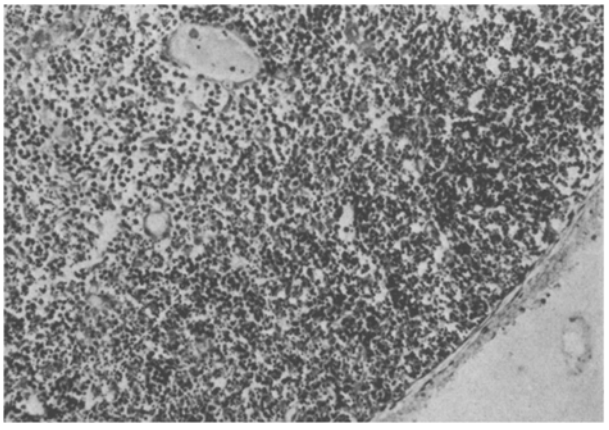

Fig. 3. Karyorrhexis in the thymic cortex of a neonatal mouse 24 h after the i.p. administration of salivary gland homogenate. ×40.

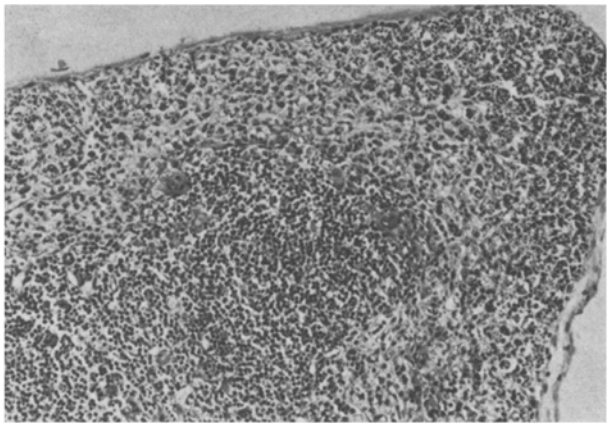

Fig. 4. Thymic cortex devoid of intact lymphocytes 48 h after the i.p. administration of salivary gland homogenate. ×40.

lymphocytes. It appears that they disintegrate and migrate into the medulla[12]. The 2 cell compartments concerned correspond to the cortisol-sensitive and cortisol-resistant thymocyte subpopulation[13]. Under the influence of salivary gland homogenate, the cortisol-resistant thymocytes seem to migrate into the medulla at a faster rate. This is in agreement with the finding that cortisol-sensitivity in mice is age-dependent and not only is cytotoxicity different but also the rate of migration. It is not clear whether the various factors demonstrated (e.g. the epidermal growth factor) are present exclusively in the salivary gland, or if they only occur there to a greater extent[15].

On the one hand, thymic tissue proliferates after removal of the submandibular glands of adult mice; on the other hand, salivary gland homogenate has a suppressive action on the thymus in both adrenalectomized and gonadectomized mice, showing that the atrophic effects were not mediated by these organs[8]. Thus it cannot be ruled out that there is a substance in the salivary gland which has a cortisol-like effect and which regulates the cell kinetics of the thymus.

12 I. L. Weissmann, J. exp. Med. *137*, 504 (1973).
13 M. Schlesinger and E. Israël, Cell Immun. *18*, 144 (1975).
14 M. H. Claesson and V. Tjell, Acta Path. microbiol. scand. Sect. C *84*, 185 (1976).
15 M. L. Roberts, J. A. Freston and P. C. Reade, Immunology *30*, 811 (1976).

Effect of ageing on surface IgG of human peripheral lymphocytes

C. Zanussi, C. Rugarli, B. Perussia* and R. Scorza Smeraldi

Istituto di Clinica Medica IV, Padiglione Litta-Policlinico dell'Università, via Francesco Sforza 35, I-20122 Milano (Italy), 26 November 1976

Summary. An increase in the percentage of IgG bearing peripheral blood lymphocytes is observed in aged subjects as compared with young ones. Such a finding is probably due to the presence, in 'aged' sera, of a higher concentration of immune complexes, bound to lymphocytes through their Fc or Complement receptors.

The receptor function of IgM and IgD on B-cell membrane is well assessed[1-4], whilst IgGs are commonly considered as artifacts[5-7]. This evidence casts doubt on the significance of the age-associated increase of the percentage of IgG bearing lymphocytes observed in healthy humans[8]. A brief in vitro culturing of lymphocytes (1h) is useful to clear the cells from passively attached molecules and, for instance, to free them from the bulk of membrane IgG[7] without affecting the expression of the true membrane receptors. Thus we studied the effect of a 'shedding and regeneration' treatment on peripheral lymphocytes from young and aged donors, and we controlled the effect of the incubation with blood serum on the recorded values.

Materials and methods. Peripheral lymphocytes were harvested from heparinized venous blood according to the technique quoted by Aiuti et al.[9] from 10 healthy young volunteers (less than 30 years old) and 10 aged patients suffering from minor cardiovascular complaints (more

* Present address: Ludwig Institute for Cancer Research. CH-1066 Épalinges s/Lausanne, Switzerland.

1 Membrane receptors of lymphocytes. Ed. M. Seligmann, J. L. Preud'homme and F. M. Kourilsky. North-Holland Publishing Co., Amsterdam 1975.
2 E. S. Vitetta, U. Melcher, M. McWilliams, M. E. Lamm, J. M. Phillips Quagliata and J. W. Uhr, J. exp. Med. *141*, 206 (1975).
3 D. Rowe, K. Hug, L. Forni and B. Pernis, J. exp. Med. *138*, 965 (1973).
4 E. S. Vitetta, J. Forman and J. R. Kettman, J. exp. Med. *143*, 1055 (1976).
5 E. S. Vitetta and J. W. Uhr, Science *189*, 964 (1975).
6 S. M. Fu, R. J. Winchester and H. G. Kunkel, J. exp. Med. *139*, 451 (1974).
7 R. J. Winchester, J. B. Winfield, F. Siegal, P. Wernet, Z. Bentwich and H. G. Kunkel, J. clin. Invest. *54*, 1082 (1974).
8 H. A. Del Pozo Perez, J. Prieto Valtuena, M. I. Gonzales Guilabert and R. Velasco Alonso, Biomedicine *19*, 340 (1973).
9 F. Aiuti, J.-C. Cerottini, R. R. A. Coombs et al., Clin. Immun. Immunopath. *3*, 584 (1975).