the lack of an immunopositive reaction in cell bodies may only indicate that the sensitivity of the technique is insufficient. Further studies are clearly necessary to establish the localization of the entire somatostatin neurons. Such studies should also include other tissues such as spinal ganglia, thus persuing the possibility that the somatostatin containing nerves are of sensory nature. Preliminary studies, e.g. on the pancreas, thyroid gland, salivary gland, liver, kidney, adrenals, spleen and thymus, have not demonstrated somatostatin positive nerves in these tissues. Thus, so far, somatostatin-containing nerves seem to be present only in the hypothalamus[9-11], the posterior pituitary[18] and the intestine.

The presence of somatostatin or a somatostatin-like peptide in probable nerves with a clearly different distribution from those of the Substance P-containing nerves (and of cholinergic and adrenergic nerves) indicates the presence of a further type of nerve in the gut. The functional rôle of these nerves is at present unknown. Quantitatively, these new nerves, however, seem to be of less importance than, for instance, the Substance P-containing systems. Some somatostatin-containing nerves seem related to ganglion cells, others are found between smooth muscle cells, whereas those in the lamina propria could influence blood vessels. It may be pointed out that somatostatin in the hypothalamus inhibits growth hormone secretion[8] and that in the pancreas this peptide inhibits insulin and glucagon secretion[21-28]. Finally, it should be emphasized that, although the immunoreaction is specific in the sense that it is abolished by pretreatment of the somatostatin antiserum with somatostatin, the possibility of a cross reaction with a similar peptide cannot be excluded[29].

Summary. Antibodies to somatostatin, a recently isolated hypothalamic peptide inhibiting growth hormone release, were used in immunohistochemical studies on the gastrointestinal tract. Somatostatin containing cells in the stomach, and somatostatin-containing nerves in the small and large intestine, could be demonstrated. These findings give evidence of a new type of nerve in the gut.

T. HÖKFELT, O. JOHANSSON, S. EFENDIC, R. LUFT and A. ARIMURA

Department of Histology, Karolinska Institute, and Department of Endocrinology and Metabolism, Karolinska Hospital, S-104 01 Stockholm (Sweden), and The Veterans Administration Hospital and Tulane University School of Medicine, New Orleans (Louisiana, USA), 14 April 1975.

[21] K. G. M. ALBERTI, S. E. CHRISTENSEN, J. IVERSEN, N. J. SAYER-HANSEN, N. J. CHRISTENSEN, A. PRANGE-HANSEN, K. LUNDBAECK and H. ØRSKOV, Lancet *2*, 1299 (1973).
[22] F. P. ALFORD, S. R. BLOOM, J. D. N. NABARRO, R. HALL, G. M. BESSER, D. H. COY, A. J. KASTIN and A. V. SCHALLY, Lancet *2*, 974 (1974).
[23] S. EFENDIC and R. LUFT, Acta endocr., Copenh. *78*, 510 (1975).
[24] S. EFENDIC and R. LUFT, Acta endocr., Copenh. *78*, 516 (1975).
[25] D. J. KOERKER, W. RUCH, E. CHODECKEL, J. MALMER, C. J. GOODNER, J. ENSINCK and C. C. GALE, Science *184*, 482 (1974).
[26] C. H. MORTIMER, W. M. G. TUNBRIDGE, D. CARR, L. YEDMASS, T. LIND, D. H. COY, S. R. BLOOM, A. J. KASTIN, C. N. MALLINSON, G. M. BESSER, A. V. SCHALLY and R. HALL, Lancet *1*, 697 (1974).
[27] S. S. C. YEN, M. T. SILER and G. W. DEVANE, New Engl. J. Med. *290*, 934 (1974).
[28] H. SAKURAI, R. DOBBS and R. H. UNGER, J. clin. Invest. *54*, 1395 (1974).
[29] This study was supported by grants from the Swedish Medical Research Council (Nos. 14X-2887 and 19X-3411), from Magnus Bergvalls Stiftelse and from Greta and Harald Jeanssons Stiftelse. The skillful technical assistance of Miss. A. NYGÅRDS is gratefully acknowledged. The generous supply of somatostatin by Dr. R. GUILLEMIN, The Salk Institute, San Diego, California/USA, is gratefully acknowledged.

Effect of *Mycobacterium bovis* and *Mycobacterium tuberculosis* on the Take and Survival of Chickens with Transplanted MC-29 Hepatoma

The therapeutic effect of *Mycobacterium bovis* (BCG) and of mycobacterial cell walls has been demonstrated in several experimental tumor systems[1-11]. The use of BCG in the therapy of human tumor patients has also given promising results in a number of cases[12]. No data, however, are available about the effect of BCG in experimental transplantable tumor systems of a certain virus origin and having an epithelial character.

The present investigation was designed to test the effect of BCG (Paris strain) and of *Mycobacterium tuberculosis* (W-115 strain[13,14]) on a transplantable chicken tumor system of a proved virus origin.

Newly hatched Hunnia hybrid chickens kept on a standard diet were used. They were selected for the different groups at random and were controlled daily. A post mortem examination of every chicken was performed.

The transplantable MC-29 hepatoma established from a primary hepatoma induced by the oncogenic MC-29 RNA virus isolated in Bulgaria[15] was used as tumor system. We transplant this tumor every 14th day i.m. and s.c. and

Table I. Comparative tumor take and tumor growth of chickens after transplantation of MC-29 hepatoma with and without BCG

Series of experiment	Treatment	No. of animals	Tumor at the site of transplantation	Mean $\frac{\text{tumorweight}}{\text{bodyweight}} \times 100$
1	Only tumor	11	10/11[a]	9.3 ± 8.3[b]
	Living BCG + tumor at the same site	12	3/12	2.1 ± 1.2
2	Only tumor	10	10/10	10.0 ± 2.2
	Heat killed BCG + tumor at the same site	15	12/15	16.7 ± 4.7

[a] Number of animals with tumor/number of animals tested. [b] Standard error of the mean.

Table II. Comparative survival of chickens after transplantation of MC-29 hepatoma with and without living BCG or W-115

Series of experiment		Treatment	No. of animals	Survival (days)	
3	a)	Only tumor	12	20 ± 5.5 [a]	
	b)	Living BCG + tumor at the same site	13	50 ± 26.8	p (a = b) < 0.005 [b]
	c)	Living W-115 + tumor at the same site	12	59 ± 32.5	p (a = c) < 0.005 [b]

[a] Standard error of the mean. [b] Student's t-test.

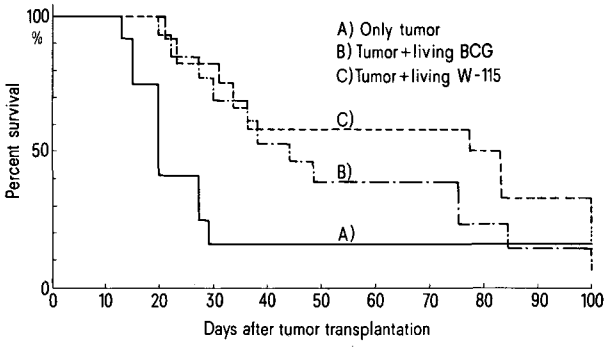
have a positive take in 90–100%. The tumor grows rapidly and on reaching the weight of about 1/5th–1/10th of the body weight, kills the chickens within 3 weeks. Morphologically the tumor cells show a hepatocellular character and virus particles can be seen electronmicroscopically in each tumor[16].

Saline suspensions of 14-day-old Sauton cultures of strains BCG-P and W-115 in a concentration of 20 mg/semi-dry weight/ml were prepared. Groups of chickens obtaining a s.c. tumortransplant in 0.2 ml prepared in PBS by a standard homogenization method, and similar groups obtaining the same tumortransplant together with 4 mg BCG or W-115, were compared by two methods: 1. The animals were killed the 14th day after transplantation and the gross changes were compared (Table I). 2. Survival of the animals of 2 groups was compared (animals alive the 100 th day after transplantation were sacrificed) (Table II and Figure).

Table I shows that 10 out of 11 chickens developed tumor at the site of transplantation in the group of chickens getting no BCG. On the contrary, only 3 out of 12 chickens developed tumor if it was given together with living BCG. The mean value of the tumor weights of the group obtaining no living BCG is markedly greater than that of the BCG-treated group. It can also be seen that application of killed BCG had no effect, either on the tumor take or on the tumor growth.

Table II and the Figure show that giving living BCG or W-115 together with the tumor transplant significantly prolongs the survival of the chickens in comparison to the group obtaining a tumor transplant without BCG or W-115.

The results of this study show that living BCG or W-115 given together with a transplant of MC-29 hepatoma inhibit (but do not prevent) the growth of the tumor transplant in newly hatched Hunnia hybrid chickens. The effect can be explained 1. by a nonspecific immunestimulation, or 2. by a direct activation of the host macrophages by BCG, thus destroying the tumor cells by the lysosomal enzymes of the macrophages. Further experiments are needed to test these hypotheses.



Comparative survival of chickens after tumor transplantation.

Zusammenfassung. Die gleichzeitige Verabreichung von BCG und von MC-29 Hepatoma-Transplantaten an frisch geschlüpfte Hühnchen, vermindert das Auftreten progressiv wachsender Tumoren. Sowohl BCG als auch lebendes *Mycobacterium tuberculosis* (Stamm W-115) bewirken unter gleichen Bedingungen eine Verlängerung der Überlebenszeit der Tiere.

I. Földes[17] and Ágnes Gyuris

Microbiological Research Group of the Hungarian Academy of Sciences, Pihenö ut. 1, 1529 Budapest (Hungary), 29 November 1974.

[1] L. G. Bartlett, B. Zbar and H. J. Rapp, J. natn. Cancer Inst. *48*, 245 (1972).
[2] B. Zbar, T. R. Meyer, I. Azuma and H. J. Rapp, J. natn. Cancer Inst. *52*, 1571 (1974).
[3] P. Minden, M. Wainberg and D. W. Weiss, J. natn. Cancer Inst. *52*, 1643 (1974).
[4] E. Yarkoni, L. Wang and A. Bekierkunst, J. Infect. Immun. *9*, 977 (1974).
[5] R. Turcotte and M. Quevillon, J. natn. Cancer Inst. *52*, 215 (1974).
[6] D. M. Jacobs and M. L. Kripke, J. natn. Cancer Inst. *52*, 219 (1974).
[7] T. J. Meyer, E. E. Ribi and B. Azuma, J. natn. Cancer Inst. *52*, 103 (1974).
[8] I. Azuma, E. E. Ribi, T. J. Meyer and B. Zbar, J. natn. Cancer Inst. *52*, 95 (1974).
[9] E. Yarkoni, A. Bekierkunst, J. Asselinean, R. Toubiana, M. J. Toubiana and E. Lederer, J. natn. Cancer Inst. *51*, 717 (1973).
[10] D. H. Lavrin, S. A. Rosenberg, R. J. Connor and W. D. Terry, Cancer Res. *33*, 472 (1973).
[11] M. R. Schinitsky, L. R. Hayman, A. A. Blazkovec and P. M. Burkholder, Cancer Res. *33*, 659 (1973).
[12] M. J. Mastrangelo, Y. H. Kim, R. S. Bornstein, D. O. Chee, H. L. Sulit, J. W. Yarbro and R. T. Prehn, J. natn. Cancer Inst. *52*, 19 (1974).
[13] Gy. J. Weissfeiler, Proc. Microbiol. Res. Group Hung. Acad. Sci. *1*, 51 (1966).
[14] Gy. J. Weissfeiler and V. Karasseva, Am. Rev. resp. Dis. *92*, 870 (1965).
[15] X. Ivanov, Z. Mladenov, S. Medyalkov, T. G. Todorov and M. Yakimov, Bull. Inst. Path. comp. Anim. Domest. Acad. Sci. Bulg. *10*, 5 (1964).
[16] A. J. Langlois, K. Lapis, R. Ishizaki, J. W. Beard and D. P. Bolognesi, Cancer Res. *34*, 1457 (1974).
[17] We thank Professor K. Lapis for discussions and suggestions and B. Gergely for his technical assistance.