G-6-PD inhibition could be demonstrated for this second bowel tumour system.

The methods for transplantation and chemotherapy of GW-39, as well as for spectrophotometric assay of G-6-PD activity, have been described elsewhere[11–13]. Table I presents the results of 2 experiments using well tolerated doses of actinomycin C (Sanamycin®, Bayer). Tumour inhibition has been calculated by comparing mean increase in tumour size, based on the first measurement of tumour volume 6–7 days after transplantation, between treated and control groups of tumours on the day of enzyme extraction. All enzyme determinations were performed between 24 and 36 h after the last i.p. injection of actinomycin. Enzyme activity is expressed in IU/mg total nitrogen. As can be seen, tumour inhibitory doses of actinomycin once more significantly inhibit G-6-PD activity. Liver enzyme levels of the same animals, however, again showed no significant change due to actinomycin therapy (Table II), thus reconfirming the tumour-specificity of this effect.

Table I. G-6-PD activity of GW-39 tumours after actinomycin C therapy

Daily dose mg/kg	Days treated	No. of tumours assayed	Tumour G-6-PD activity (± S.D.)	Significance[a] (P) treated vs. controls	Tumour growth inhibition (%)
0.06	7	6	37.9 ± 4.6	< 0.001	47
controls		5	86.7 ± 12.2		
0.08	7	5	55.9 ± 13.1	< 0.005	59
controls		5	89.6 ± 14.4		

[a] P according to Student's t test.

Table II. Hamster liver G-6-PD activity after actinomycin C therapy

Daily dose mg/kg	No. of livers assayed	Liver G-6-PD activity (± S.D.)	Significance (P) treated vs. controls
0.06	3	27.5 ± 8.8	0.1
controls	3	39.8 ± 3.1	
0.08	4	57.2 ± 16.4	0.9
controls	4	55.6 ± 7.7	

These results present additional evidence for the correlation between the high anti-tumour activity of actinomycin in such human colonic cancers and tumour G-6-PD inhibition. No cause-and-effect relationship, however, can be postulated at this time. Indeed, a primary effect of actinomycin on DNA-directed synthesis of RNA[14,15] might secondarily affect this enzyme, a point which should be clarified by testing other template-inhibiting active and inactive anti-tumour compounds[16].

Zusammenfassung. Die Aktivität der Glukose-6-phosphat-Dehydrogenase (G-6-PD) wurde in Extrakten des heterotransplantierten Humantumors GW-39 nach cytostatischer Therapie mit Actinomycin C untersucht. Es kam zu einer signifikanten Hemmung der G-6-PD-Aktivität in den Tumoren, während die Enzymaktivität in der Leber der tumortragenden Wirtstiere (Hamster) unbeeinflusst blieb. Diese Ergebnisse entsprechen unseren, an einem histopathologisch ähnlichen Humantumorsystem (H.Ad. No. 1) früher erhobenen Befunden und bestätigen damit den tumorspezifischen Enzymeffekt der Behandlung mit Actinomycin C.

D. M. Goldenberg, S. Witte and W. Ernstberger

Cancer Research Laboratory, Department of Medicine, University Clinics, University of Erlangen-Nürnberg, 852 Erlangen (Germany), 14th June 1967.

[14] J. Hurwitz, J. J. Furth, M. Malamy and M. Alexander, Proc. natn. Acad. Sci. U.S.A. *48*, 1222 (1962).

[15] I. H. Goldberg, M. Rabinowitz and E. Reich, Proc. natn. Acad. Sci. U.S.A. *48*, 2094 (1962).

[16] This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft.

Micturition Behaviour of Neonatally Testosterone Treated Female Dogs

It is generally accepted that the pattern of activity of the hypothalamus-hypophysis axis can be changed or shifted to the so-called male type – lessened luteinizing hormone secretion and absence of cycles – when testosterone is injected during the first days of life. Female rats so treated are anovulatory and vaginal estrus is maintained[1].

This paper deals with another type of masculinization, through hetero-sexual change of the nervous centres controlling behaviour: the wellknown adult postural pattern of the dog at micturition. We have already shown (Martins and Valle[2–6]) that male dogs when early castrated (28–64 days of age) maintained through life their peculiar infantile attitude at micturition (IP)[7]. However, they shifted to the adult normal type of lifting one of the hind legs if treated with testosterone propionate (TP). We have also observed that infantile dogs treated with this hormone shifted to the adult male posture (AMP) earlier than the normal male controls. It is interest-

[1] C. A. Barraclough, Endocrinology *68*, 62 (1961).

[2] Th. Martins and J. R. Valle, Mems Inst. Butantan *16*, 237 (1942).

[3] Th. Martins and J. R. Valle, C. r. Séanc. Soc. Biol. *141*, 620 (1947).

[4] Th. Martins and J. R. Valle, C. r. Séanc. Soc. Biol. *141*, 623 (1947).

[5] Th. Martins and J. R. Valle, J. comp. physiol. Psychol. *41*, 301 (1948).

[6] Th. Martins and J. R. Valle, Abstracts III. Int. pharmac. Congr. (Sao Paulo) *364*, 144 (1966).

ing to note that this same result may be obtained with estradiol benzoate (EB) if the hormonal treatment starts on the 3rd day of life.

The normal female pattern at micturition also exhibited by infantile and castrated bitches, was unchanged after TP, even when the treatment started at 39–50 days of age. In only one case the AMP was eventually exhibited by a treated animal whose mother had received injections of TP just before fertilization. Complete male pattern of behaviour at micturition, however, was shown by female dogs receiving, from the third day of life, a series of TP injections over several weeks. This change to the AMP occurred earlier than in the normal control males and persisted several months after cessation of the treatment.

Emphasis is given now to the results we have recently obtained through early and short treatment of female puppies with TP (Table).

The difference of this last neonatal series is that the number of injections, instead of being continued for several weeks, was limited to 1 or 4, from the 1st to the 12th day of life.

The female dogs of litter D, 3 years old now, maintain the complete AMP at micturition from their 8 months of age. Castration of one of them, 6 months ago, did not interfere with her male behaviour when urinating (Figure). It is interesting to note that the removed ovaries contained newly formed corpora lutea.

In all experimental groups, no feminization of the micturition behaviour was attained in males as a consequence of EB treatment; on the contrary, the female hormone even induces precocious and complete AMP.

Micturition behaviour and genital development of neonatally hormone treated puppies

Litter	Puppies			Treatment[a] No. of injections and dosage	Remarks
	Total	Male	Female		
A	2	1	1	1 ♂: 1 × 0.5 mg estradiol benzoate	Unchanged male type of behaviour at micturition (AMP).
				1 ♀: 1 × 5.0 mg testosterone propionate	Penisoid clitoris. Unchanged female type of behaviour.
B	6	4	2	2 ♂: Untreated	Normal male type of behaviour (AMP).
				2 ♂: 1 × 0.5 mg estradiol benzoate	Unchanged male type of behaviour (AMP).
				2 ♀: 1 × 4.0 mg testosterone propionate	Penisoid clitoris. One bitch eventually exhibited the male type of behaviour
C	6	–	6	6 ♀: Untreated	Normal female type of behaviour.
D[b]	6	4	2	4 ♂: Untreated	Normal male type of behaviour (AMP).
				2 ♀: 4 × 4.0 mg testosterone propionate	Penisoid clitoris. Both females presented the complete male type of behaviour (AMP).

[a] Oil solution of the gonadal hormones s.c. injected the first day of life except for the female puppies of litter D which have received the male hormone the first, 4th, 8th and 12th day of life and a total dose of 16 mg each. [b] Four days before parturition the mother received s.c. 25 mg of testosterone propionate.

In normal males or masculinized females, the behaviour was uninfluenced by anaesthesia of the nostrils with xylocaine emulsion[8]. The postural troubles eventually observed in our experiments seem to depend on ataxic phenomena induced by the drug.

The data here presented may be taken as an additional contribution to the problem of masculinization of brain centres following testosterone, when administered during a critical plastic period of organization. They have many points of contact with the reports on masculinization of the hypothalamus-hypophyseal axis in neonatally injected females.

Confirming what we have suggested in 1948, it seems that testosterone may function as an organizer or activator of genetically predetermined nervous centres during embryonic differentiation or in the early post-natal period.

Adult female dog D2, born 26th May 1964, s.c. injected with 4 mg of testosterone propionate the first, 4th, 8th and 12th day of life. Photo taken by the end of November 1966. The complete male pattern of behaviour at micturition was maintained even after castration when $2^1/_2$ years old. At right her mother exhibiting the normal female posture while urinating.

Résumé. Nous avons montré que si l'on traite des chiens nouveau-nés de sexe féminin par la testostérone, même durant peu de temps, on peut changer leur attitude femelle de miction qui devient, après la puberté, du type charactérisant le mâle adulte, avec élévation d'une patte postérieure. Il semble que la testostérone, avant ou peu après la naissance, joue un rôle dans l'organisation ou l'activation des centres nerveux génétiquement prédéterminés et en relation avec les voies réflexes liées au comportement de l'animal adulte selon son sexe.

TH. MARTINS and J. R. VALLE

Department of Physiology, Faculdade de Medicina, Universidade Federal do Rio de Janeiro and Department of Biochemistry and Pharmacology, Escola Paulista de Medicina, Sao Paulo (Brazil), 10th May 1967.

[7] In the infantile male pattern at micturition (IP) there is abduction, a slight extension and sometimes a little flexion of the hind legs. When these are extended a slight projection of the trunk is also frequently observed. The pattern for infantile and adult females is the same (cf. [5] p. 305).

[8] J. FREUD and I. E. UYLDERT, Acta brev. neerl. Physiol. *166*, 49 (1948).