Table II. Percentage of viable cells in trypan-blue cytotoxicity tests performed with NM or XM cells exposed to rabbit anti-embryonic mouse serum

Type of marrow used for additional absorption	Type of marrow tested	Percentage of viable cells						
		No serum	serum dilutions					
			1/2	1/4	1/8	1/16	1/32	1/64
NM	NM	82	84	86	82	—	—	—
	XM	80	61	57	63	60	76	84
XM	NM	82	73	82	83	—	—	—
	XM.	80	69	79	75	—	—	—

The serum was absorbed 4 times with different adult mouse tissues and, in addition, with either NM cells or XM cells.

These data suggest that the rabbit antiserum produced against embryonic mouse tissues contains antibodies which are specifically active against a part of the cell population, present in the bone marrow of irradiated mice but not in the marrow of untreated mice. Since X cells are immature in character and are present in new-born animals, it is tempting to assume that X cells which accumulate in the marrow after irradiation have specific embryonic antigens and therefore represent this fraction. However, it cannot be excluded that some part of the cell population present in irradiated marrow, X cells or another cell type, is more sensitive to certain antibodies than are unirradiated marrow cells. It is unlikely that H-2 antibodies caused the observed effect, since such antibodies are not easily detectable in xenogeneic sera[8]; the absorption procedures used would eliminate such antibodies; and, furthermore, cells from irradiated and normal bone marrow have been shown to have a similar sensitivity to the cytotoxic effect of such antibodies[9]. It is expected that further tests using immunofluorescence, and cell separation techniques will distinguish between the different possibilities discussed above[10].

Résumé. Un antisérum dirigé contre les tissus embryonnaires de la souris contient des anticorps capables de détruire sélectivement une population de cellules présente dans la moelle qui régénère après une irradiation sublétale. On peut supposer que la population détruite correspond à un type particulier de cellules lymphoïdes immatures (cellules X) qui caractérise la régénération médullaire de la souris irradiée.

J. Haot[11], Erna Möller[12], K. Hiesche[13] and L. Révész[13]

Institut d'Anatomie Pathologique de l'Université, 1, rue des Bonnes Villes, B-4000 Liège (Belgium); Division of Immunobiology, Wallenberg Laboratory, Lilla Frescatit and Department of Tumor Biology, Karolisnka Institutet, Stockholm (Sweden),
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[11] Institut d'Anatomie Pathologique, Université de Liège, Belgium.
[12] Division of Immunobiology, Wallenberg Laboratory, Lilla Frescati.
[13] Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden.

Possible Role of Growth Hormone in the Stimulation of the Thymus of Rats Following Irradiation of the Head

Exposure of the head only – with the thymus and spleen completely shielded – with doses of X-rays between 150–2000 R produces biochemical changes in the lymphoid organs of young rats[1,2]. The first effect – evident at 4 h post-irradiation and reaching a maximum at 24–48 h – is a marked increase in the rate of incorporation of tritiated thymidine in the DNA of thymus cells. In other lymphoid organs, notably the spleen, head irradiation does not affect the synthesis of DNA[2].

The object of this investigation is to examine the mechanism by which irradiation with X-rays of the head only influences DNA metabolism in the thymus. The neuro-endocrine system exerts both a negative and a positive control over the thymus. By releasing ACTH cell division is reduced via the intermediary of cortisone[3,4], on the other hand there is evidence that other pituitary hormones, growth hormone, parathyroid hormone and vasopressin may stimulate proliferation of lymphoid cells in the thymus[5–10] and growth hormone was found to be thymotropic[11]. Consequently stimulation of the thymus following head irradiation might be caused either by reducing the output of ACTH or by increasing the supply of growth hormone. In the literature data can be found in support of either mechanism after head irradiation. Mirand and Hoffman[12] and Hameed and Haley[13] deduce that the release of ACTH is reduced for a short period and Mosier and Jansons[14] find an increase in pituitary content of growth hormone. The effect of head irradiation on bilaterally adrenalectomized rats was, therefore, investigated so as to distinguish between these two mechanisms.

A further question is whether the target organ responsible for the increase in thymidine incorporation by head irradiation is the pituitary or the hypothalamus. There is

In vivo incorporation of thymidine into DNA of cells in the thymus of weanling rats following different treatments[a]

Treatment[a]	DNA content (mg)	Incorporation of radioactive thymidine into DNA of the cells in the thymus (cpm/mg of DNA)[b]
None (control) (3)	1.5±0.73	250± 71
Head irradiation (5)	1.0±0.16	1300± 223
Adrenalectomy (6)	3.7±0.80	1100± 269
Adrenalectomy and head irradiation (9)	1.5±0.61	2500± 837
Epinephrine (4)	1.9±0.49	2030± 584
Serotonin (6)	1.6±0.37	776± 280
Growth hormone (7)	1.1±0.42	13000±2790

[a] Values are means±S.D. of results. Number of rats used in each experiment is in parentheses. [b] Activity and weight of thymus measurements were carried out 48 h after treatment, except for treatment with growth hormone when it was 12 h.

some evidence[15] that hypothalamic centres are affected almost immediately after exposure to X-rays and this raises the possibility that radiation alters the output of the neurosecretions which control the function of the adenohypophysis. The effect on the thymus of pharmacologically active agents with central activity was, therefore, compared with that of head-irradiation. The data to be presented is consistent with the hypothesis that head-irradiation increases neurosecretions from hypothalamic centres which in turn increase the output of a growth stimulating factor for the thymus.

Material and methods. Male random-bred Wistar rats aged 3–4 weeks and weighing between 30 to 45 g were used throughout.

Irradiation with 1000 R of 250 kV X-rays confined to the head only was carried out as previously described[2]. The dose of X-rays to the thymus was less than 10 R. The rats were killed 48 h after irradiation.

Adrenalectomy was performed bilaterally by the dorsal route under ether anaesthesis. All extirpated glands were kept in a moist chamber until they could be examined under the dissection, or if the capsule on both sides showed tears, the animal was not used. Sham adrenalectomy was performed in exactly the same way, and both adrenals were exposed but not touched 30 min after operation. The rats were head irradiated with 1000 R, and killed 48 h later.

Pharmacologically active agents and hormones. The rats were injected intraperitoneally. a) Epinephrine (Teva) 50 ng per rat; the rats were killed 48 h later. b) Serotonin (N.B.C) 25 ng per rat; 1 injection at time zero and a second one after 24 h; killed 48 h after first injection. c) Growth hormone (human), Beilinson (batch No. 14), 300 ng per rat; the rats were killed 12 h later.

Analysis of the thymus. 15 min before killing, the rats were injected i.p. with 1 μCi/g body weight of uniformly ^{3}H-labelled thymidine (Amersham) with a specific activity of 5 Ci/mmole. Immediately after killing the thymuses were removed, and weighed.

The thymus was homogenized in 5 ml of ice-cold distilled water at 4°C using a glass homogenizer fitted with a Teflon plunger, rotated at about 2000 rpm for 1 min and then immersed in a salt-ice mixture at −10°C. The frozen homogenate was cooled to −70°C and then thawed. DNA was extracted from the homogenate according to the procedure of CRADDOCK[16] and counted by scintillation in a Tri-carb Packard Counter. The DNA content of the homogenate was determined colourimetrically by the DISCHE method[17].

Results and discussion. The Table shows that both head irradiation and adrenalectomy lead to a dramatic increase in the rate of incorporation of exogenous thymidine into the DNA of the cells in the thymus. The effect of adrenalectomy might be ascribed to stimulation of the thymus due to removing the restraint exerted on the thymus by adrenal corticoids. The possibility that the stimulation by head irradiation can be ascribed to a suppression of corticoid release because head irradiation suppress the release from the pituitary of ACTH is improbable because head irradiation also increases thymidine incorporation in the thymus of adrenalectomized animals. The results of this latter experiment suggest that head-irradiation causes increased release of a pituitary hormone which stimulates the thymus. The finding that both epinephrine and serotonin increase incorporation of thymidine could also best be explained by the release, as a result of hypothalamic action, of a thymus stimulating factor. An obvious candidate for such a factor is growth hormone[14], and as can be seen from the Table, growth hormone causes a rapid and very great increase in thymidine incorporation in the thymus.

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We have not carried out any experiments which would allow us to determine whether the effect of adrenalectomy on DNA metabolism is due to the absence of corticoids, the stimulation of the pituitary or an effect on the hypothalamus mediated by releasing factors.

It must be emphasized that these experiments have been performed with young rats which are growing rapidly, and do not necessarily apply to adult animals. The hypothalami of young rats contain much more growth hormone releasing activity than the hypothalami of adults.

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Zusammenfassung. Die Röntgenbestrahlung des Kopfes junger Ratten verursacht eine vermehrte DNS-Synthese im Thymus, was mit der Freisetzung von Wachstumshormon-ähnlichen Substanzen in Zusammenhang gebracht wird.

D. Maor and P. Alexander[18]

Department of Human Microbiology,
Tel-Aviv University,
Tel-Aviv (Israel) and
Chester Beatty Research Institute,
Institute of Cancer Research,
Cliffton Avenue, Belmont,
Sutton (Surrey, England),
13 December 1971.

Competitive Inhibitors of Neurohypophyseal Hormones on Adenylate Cyclase from the Toad Urinary Bladder[1,2]

The three-dimensional structure recently proposed for oxytocin in solution[3] provides a model to correlate the conformation of the molecule with various other aspects of the hormone, e.g., its evolution, biologic activity, antigenicity, immunochemical reactivity, enzymic degradation, etc[4-6]. In the 'cooperative model' of oxytocin[4] (Figure 1) the chemically active groups (3 carboxamide groups and the phenolic hydroxyl group), and the acyclic prolyl-leucylglycinamide moiety, are oriented towards the same side of the 20-membered cyclic component of the hormone. Such an arrangement results in a hydrophilic region, which we believe to be important for the expression of the inherent catalytic activity (i.e. the 'intrinsic activity'[7]) of the hormone[4-6]. Certain structural modifications in this hydrophilic region, particularly if hydrophobic in character, might be anticipated to reduce the catalytic activity of the peptide. An analog in which the modified group(s) were sufficiently large and properly oriented, but did not interfere with the binding of the hormone to the receptor, could be expected to be an inhibitor of the hormone. Structural changes in positions 2, 4 and 9 of oxytocin appear particularly suitable for converting the hormone into an inhibitory analog. The extensive studies in whole animal and various organ preparations with the inhibitor [2-*O*-ethyltyrosine]-oxytocin, and other neurohypophyseal analogs in which the phenolic hydroxyl group is either alkylated or substituted, may be cited as examples (see ref.[8] for a recent summary). The inhibitory property of [2-*O*-ethyltyrosine]-oxytocin is also found in the intact toad urinary bladder[9] and in subcellular preparations of toad bladder epithelium[10], while the neurohypophyseal hormones per se enhance the permeability of the amphibian bladder to water and to certain small molecules[11,12]; strong evidence has accumulated indicating that this hormone-induced process is mediated by cyclic 3′,5′-AMP[10,13,14].

It has also been found that neurohypophyseal hormone analogs which have in common a leucine substitution for the glutamine residue in position 4 of oxytocin or for the serine residue in position 4 of mesotocin, i.e. [4-leucine]-oxytocin[15-17], [2,4-dileucine]-oxytocin[18], and [4-leucine]-

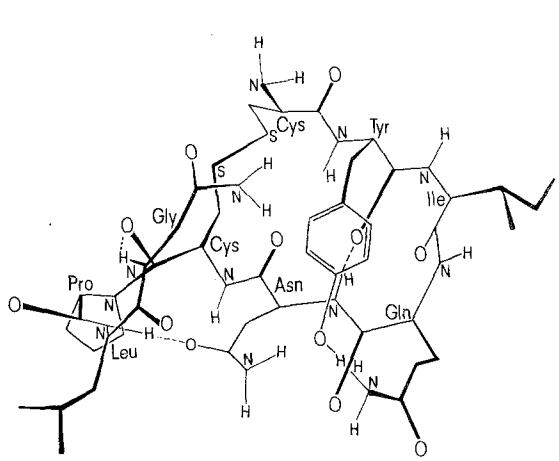

Fig. 1. Schematic representation of the *hypothetical model of the biologically active conformation of oxytocin* ('Cooperative model').

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