

cutaneously for 1 week. 11 rats were kept 1 week as untreated controls to study the effect of surgical denervation alone on the weight of the submaxillary gland. Atropine as the sulphate was given at intervals of 6 or 12 h and Hoechst 9980 (piperidino-ethyl-diphenyl-acetamid hydrochloride) at intervals of 6 h. The doses of the drugs were gradually increased during the period of treatment. In the group of rats treated with atropine at intervals of 12 h the dose was increased from 5–20 mg, total amount 180 mg; in the other atropine-treated group the dose was augmented from 1–5 mg, total dose 84 mg. The dose of Hoechst 9980 was increased from 0.5–2 mg in one group and from 1–5 mg in another, total amounts 36 and 84 mg respectively. All rats were killed by cervical dislocation after 1 week 4–6 h after the last injection. The submaxillary glands were carefully dissected, cleaned and weighed (wet weight). The dry weight of the glands was estimated after heating to 105–110°C for 48 h.

Surgical parasympathetic denervation was found to decrease the dry weight of the submaxillary gland by about 25% in 1 week, from 37 ± 1.5 (11)⁶ to 28 ± 1.6 (11) mg (Table). Treatment with atropine caused a similar, significant decrease in the weight of the normally innervated gland (Table). The reduction in the size of the glands can be considered to be specific in the group given 84 mg atropine but not in that given 180 mg atropine since the rats in the last group, but not in the first one, showed a marked decrease in body weight. Treatment with Hoechst 9980 had an effect similar to that of atropine on the weight of normally innervated glands when given in sufficient amounts (Table). The body weight was not affected by the doses of Hoechst 9980 used. Treatment with atropine or Hoechst 9980 did not change the weight of the parasympathetically denervated submaxillary glands. The dry weight of the glands was found to be about 25% of the wet weight in all groups.

'Pharmacological' denervation has been found to induce changes, e.g. supersensitivity to secretory agents, similar to those seen after surgical denervation in salivary glands of cats^{3,7} and rats⁴, while it does not cause a glandular atrophy. In the present investigation it was found that treatment with parasympatholytics, atropine or Hoechst 9980, reduces the weight of the submaxillary

gland of rats. It should be recalled that the doses of atropine and particularly Hoechst 9980 are markedly bigger than those used in previous experiments. The present finding agrees with the suggestion that the size of salivary glands is dependent on the organ activity¹.

Dry weight of the right and left submaxillary gland 1 week after parasympathetic denervation of the right gland in untreated rats (= control) and in animals treated with a parasympatholytic agent, atropine or Hoechst 9980

Group	Total dose (mg)	No. of rats	Dry weight (mg)		Right/left %
			Right	Left	
Control	—	11	28 \pm 1.6	37 \pm 1.5	74 \pm 2.9
Atropine	84	5	28 \pm 1.4	31.9 \pm 0.96 ^b	88 \pm 2.0 ^b
Atropine	180	7	26 \pm 2.3	30 \pm 1.8 ^b	86 \pm 4.5 ^a
Hoechst 9980	36	5	28 \pm 2.2	35 \pm 2.3	82 \pm 2.7
Hoechst 9980	84	5	27.5 \pm 0.94	31.3 \pm 0.65 ^b	88 \pm 1.3 ^c

Values are mean \pm S.E.M. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ when compared with the corresponding control value.

Zusammenfassung. Parasympathische chirurgische Denervierung führt zur Gewichtsabnahme der Speicheldrüsen; die Submaxillarisdrüse der Ratte zeigt eine Atrophie nach Behandlung mit parasympatholytischen Substanzen (Atropin oder Hoechst 9980).

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⁶ Mean \pm standard error of mean (number of observations).

⁷ B. C. R. STRÖMBLAD, *Acta physiol. scand.* 36, 47 (1956).

Action of Thalidomide on Endosteal Ossification in the Pigeon

The endosteal ossification is a phenomenon which occurs naturally in female birds, depending on the ovarian cycle^{1–4}.

In the pigeon, when the ovarian follicle reaches the dimension of 4–5 mm, a spicular network begins to form, which increases in a centripetal sense from the internal surface of the diaphysis of the long bones endowed with blood-forming bone marrow. When the follicle size has reached its maximum (about 20 mm in diameter) the endosteal bone has completely invaded the cavity and the marrow is reduced to islands between the spicular network. In connection with the formation of the calcareous egg-shells, there is a rapid regression of the newly-formed bone, and a few days after the deposition the initial situation is restored and the bones present a normal red bone marrow.

This cyclic phenomenon, which has the significance of realizing a deposit of Ca to be utilized in the formation of the calcareous shell, can be provoked experimentally both in the female and in the male birds by administration of estrogens⁵.

In previous research⁶, it was observed that pigeons (*Columba livia* Gm.) which had received estrogens and thalidomide for 20 days showed, in X-ray and in histological preparations, a poorer endosteal ossification com-

¹ P. KYES and T. POTTER, *Anat. Rec.* 60, 377 (1934).

² A. KIRSCHBAUM, C. A. PFEIFFER, J. VAN HENVERSWYN, and W. U. GARDNER, *Anat. Rec.* 75, 249 (1939).

³ J. BENOIT, R. GRANGAUD, and S. SARFATI, *Bull. Histol. appl. Physiol. Path.* 18, 173 (1941).

⁴ W. BLOOM and L. V. DOMM, *Anat. Rec.* 87, 91 (1941).

⁵ C. A. PFEIFFER and W. U. GARDNER, *Endocrinology* 23, 485 (1938).

⁶ V. G. LEONE and L. RINALDI, *Rc. Accad. naz. Lincei, serie VIII*, 38, 578 (1965).

pared with that of pigeons which had been treated for the same length of time with the same doses of estrogens only: the trabeculae are more scarce, thin, fragmented and irregularly located.

In order to go deeper into the problem, I have studied the effect of thalidomide at the various stages of the formation of the endosteal bone. In 47 pigeons (25 males and 22 females) the endosteal ossification was stimulated with 0.25 mg of estradiol-dipropionate (Ovociclina CIBA) per day. To one group of these animals (12 males and 11 females) 50 mg of thalidomide were also administered per os every day. Pigeons belonging to both the test and control groups were sacrificed after 5, 10, 15 and 20 days of treatment; histological and ponderal examinations of the femurs and tibiae were carried out.

From the examination of the histological preparations and from the evaluation of the endosteal ossification (i.e. the ratio between the penetration - mean value - of the newly-formed osteal trabeculae and the mean radius of the femur cross section) it was noticed that the degree of endosteal ossification in the pigeons treated with the estrogen alone increases gradually, with a stasis between the 10th and 15th day. In the pigeons treated with estrogen and thalidomide the trend of the endosteal ossification during the first 10 days is parallel with the trend of the control pigeons but the trabeculae grow thinner and penetrate deeper into the marrow. Between the 10th and 15th day it is possible to notice a net regression of the trabeculae: as a matter of fact, after 15 days of treatment the trabeculae are confined more peripherally and appear thinner and more fragmented than at the 10th day of treatment. Thereafter, the trabeculae reform and at the 20th day they have practically reached again the centre of the marrow cavity, even though they appear to be less frequent and thinner than those of the control pigeons (Figure 1).

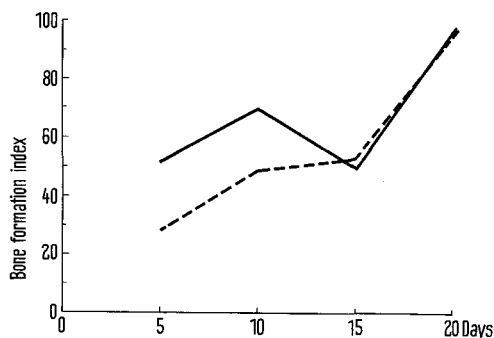


Fig. 1. ---- Treatment with estrogens; — treatment with estrogens and thalidomide.

A similar phenomenon was observed on correlating the weight of the ashes with the weight of the dry bone; the resulting ratio may be considered as an index of mineralization and, in the particular case of the bone with endosteal ossification, as a sign of the new bone formation.

While the ashes % dry bone weight ratio increases constantly in the controls (Figure 2), in the pigeons treated with estrogen plus thalidomide this value corresponds to or is even slightly higher than the controls up to the 10th day; at the 15th day there is a clear decrease of this value both in comparison with that of the control birds (at the 15th day) and with the values of the treated ones at the 10th day. After 20 days of double treatment, the mineralization appears again increased,

even though it remains distinctly below that of the controls.

Since it is known (CLAVERT)⁷ that around the 10th day of hormone treatment a certain number of osteoclasts normally appear in the endosteal bone, reaching a maximum around the 15th day, a histological control was carried out in order to establish if the remarkable absorption of the trabeculae in the same period in the presence of thalidomide can perhaps be imputed to the presence of a greater number of osteoclasts.

The examination was carried out by counting the number of osteoclasts found along a diametral line of 5 femur diaphysal sections selected at random for every animal.

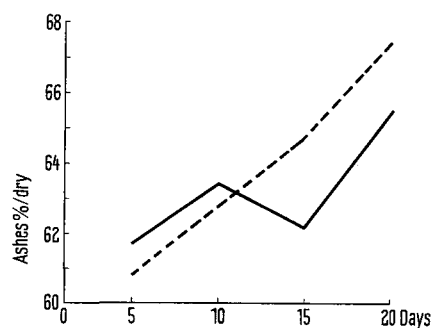


Fig. 2. ---- Treatment with estrogens; — treatment with estrogens and thalidomide.

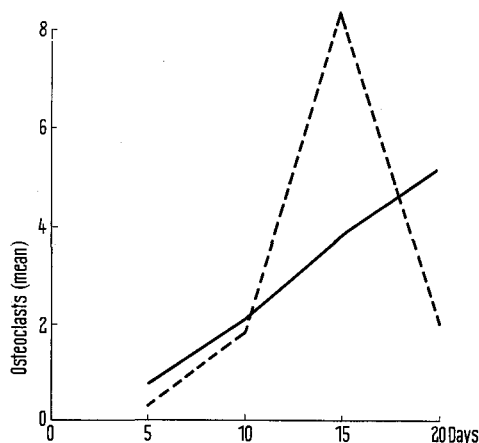


Fig. 3. ---- Treatment with estrogens; — treatment with estrogens and thalidomide.

In Figure 3 are reported the mean values of both the test and control groups, sacrificed after 5, 10, 15 and 20 days of treatment. While for the controls CLAVERT's results are clearly confirmed, for the pigeons treated with the estrogen plus thalidomide the number of osteoclasts shows a continuous increase from the 5th to the 20th day, with values clearly below those of the controls during the period of absorption.

Therefore, it is possible to exclude that thalidomide causes the absorption of the trabeculae promoting the appearance of osteoclasts.

⁷ J. CLAVERT, Bull. biol. Fr. Belg. 82, 289 (1948).

Thus, the results of this research indicate that: (1) thalidomide interferes with the formation of endosteal bone induced by means of follicular hormone in the pigeon; (2) its action is manifested not by continuous slackening in the building of the trabeculae, but rather by causing their absorption between the 10th and the 15th day of treatment; and finally that (3) the absorption is not due to an increase in the number of osteoclasts in the same period of time.

Riassunto. Ricerche condotte sulla ossificazione endostale indotta con ormone follicolare in piccioni trattati con talidomide hanno messo in evidenza che la talidomide

interferisce con l'azione ormonale; la talidomide non inibisce la formazione delle trabecole ossee, ma provoca un notevole riassorbimento delle trabecole stesse tra il 10° e il 15° giorno di trattamento; tale riassorbimento non è imputabile ad un aumento del numero di osteoclasti nel periodo corrispondente.

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Development of Hyperphagia in Male Rats Following Placement of Ventromedial Hypothalamic Lesions at Four Different Ages¹

A previous study² has shown that following ventromedial hypothalamic lesions, adult female rats showed a greater absolute food intake than did weanling animals. Relative to body weight, however, weanling and older rats ate similar amounts from the first post-operative week on. Rats lesioned during early and late adolescence showed similar food intake patterns throughout the experiment, regardless of whether food intake was expressed in g/day relative to body weight or relative to KLEIBER's 'metabolic size'³. It was suggested that age at operation and sensitivity of hypothalamic neurons might account for these findings². The present note reports on a similar study in *male* rats. Methods and experimental conditions were identical with those in the study in the female animals².

(a) *Food intake in g/day* (Figure 1). During the first post-operative week only the oldest rats with lesions (140 days) showed hyperphagia when compared with their

controls as well as with the 3 younger groups of operated rats. From the second week on, the 2 younger groups of rats with lesions (26 days and 59 days, respectively) ate similar amounts as their respective controls but the 2 older groups of rats (75 and 140 days, respectively) showed hyperphagia until the fifth week; during the sixth post-operative week all lesioned rats had plateaued to control levels. Among the operated animals, the rats lesioned at 140 days of age showed the highest food intake; during the sixth week all lesioned rats ate similar amounts.

(b) *Food intake relative to body weight* (Figure 2). The only group of rats to show a higher food intake than their controls during the first week of the experiment were the 140-day-old rats; however, they plateaued as early as the third week. Compared with their controls, rats lesioned

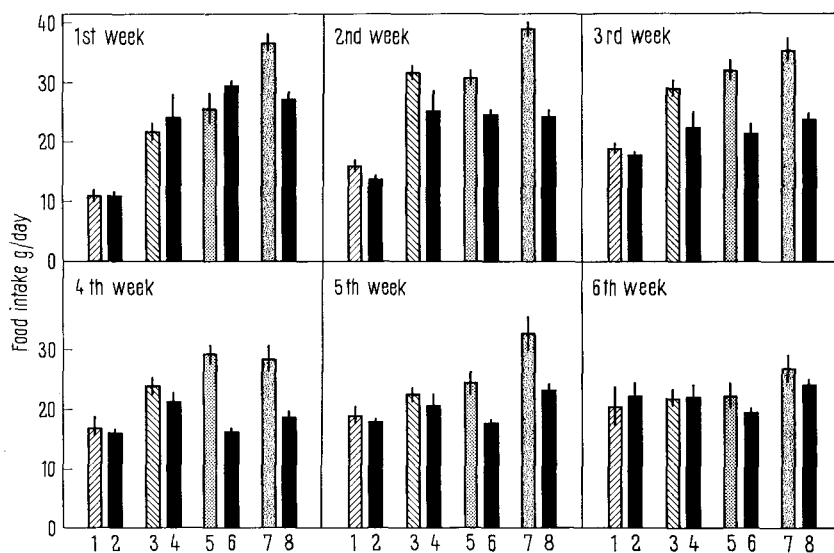


Fig. 1. Food intake in g/day of rats with hypothalamic lesions placed at the age of 26 days (group 1), 59 days (group 3), 75 days (group 5) and 140 days (group 7) and of their corresponding intact controls. Figures at bottom of diagram designate group number. Comparisons of means — First week: 7 vs 8, $p < 0.001$; second week: 1 vs 2, $p < 0.05$; 5 vs 6, $p < 0.05$; 7 vs 8, $p < 0.001$; third week: 3 vs 4, $p < 0.05$; 5 vs 6, $p < 0.01$; 7 vs 8, $p < 0.001$; fourth week: 5 vs 6, $p < 0.001$; 7 vs 8, $p < 0.01$; fifth week: 5 vs 6, $p < 0.02$; 7 vs 8, $p < 0.01$.

¹ This investigation was supported by U.S.P.H.S. Grant No. HE 06975 of the National Heart Institute.

² L. L. BERNARDIS, *Experientia* 22, 593 (1966).

³ M. KLEIBER, *Physiol. Rev.* 27, 511 (1947).