Pharmacology Biochemistry & Behavior, Vol. 5, pp. 609–615.

Involvement of the Medial Hypothalamus and the Septal Area in the Control of Food Intake and Body Weight in Geese

N. SNAPIR, M. YAAKOBI, B. ROBINZON, H. RAVONA AND M. PEREK

Department of Animal Science, Hebrew University of Jerusalem
Faculty of Agriculture, Regovot, Israel

(Received 27 January 1976)

SNAPIR, N., M. YAAKOBI, B. ROBINZON, H. RAVONA AND M. PEREK. *Involvement of the medial hypothalamus and the septal area in the control of food intake and body weight in geese.* PHARMAC. BIOCHEM. BEHAV. 5(6) 609–615, 1976. -- Intracranial injections of 6-OHDA were used to destroy the following brain areas of male geese: bilateral VMH-mamillary (BL-VMH), unilateral VMH (UL-VMH), septal area, and bilateral hypothalamic areas located dorsal, lateral, caudal and rostral to the VMH. The brain damage was nonspecific, destroying cell bodies as well as axons. The effects of these lesions were determined on food intake, body weight, abdominal adipose tissue, liver weight and fat content, and on selected endocrine gland weights. The highest food intake, obesity and liver weight and its fat content were exhibited by the BL-VMH lesioned geese. The UL-VMH and the septal lesioned geese showed moderate increase in food intake, which eventually decreased in the UL-VMH but remained constant in the septal lesioned ones. The liver fat content of the latter groups of geese was higher than the controls. No differences were found between the controls and the group of geese lesioned in the various hypothalamic areas – excluding the VMH – in all parameters measured. While no significant differences were found in pituitary, thyroid and adrenal weights among all groups of geese, a decrease in testes weight was noted in the lesioned groups of geese as compared to the controls. The interrelationships between body weight, food intake, adiposity and liver weight are discussed.

Geese Hypothalamus 6-OHDA Liver VMH

APPROPRIATE lesions in the ventromedial hypothalamic area (VMH) elicit an increase in food intake (hyperphagia) and body weight in many mammals [3, 4, 8, 12, 18, 25]. Similar results are also produced in some avian species such as domestic fowl [16, 23, 27, 28], sparrow [14] and goose [2]. The interrelationships between the hyperphagia-obesity syndrome and endoctine system, such as the gonadal or thyroid axes, have been a subject of some recent investigations in the domestic fowl using mainly the White Leghorn cockerel as the experimental model [6, 23, 24, 28, 29, 30]. Attention was given to the relation between specific locations of the lesions in the basal hypothalamus and their various physiological consequences.

Recently an attempt was made to produce fatty livers in the goose by producing hypothalamic obesity using electrolytic lesions or intracranial injections of 6 hydroxydopamine (6-OHDA) [1,2]. In view of the economic importance of fatty liver production in geese and using information already gathered on hypothalamic obesity in the domestic fowl, the purpose of this investigation was to extend knowledge on the role of the hypothalamus and the septal area in the control of food intake, body fat and liver fattening in the goose. Furthermore, the interrelationship among these various parameters appears to be of particular interest. Intracranial injections of 6-OHDA were used to produce brain lesions in various locations of the hypothalamus and septal area.

METHOD

Animals

Three to four-month-old local male domestic geese, a variety produced by crossbreeding Egyptian, Tolouse and Embden breeds, were used for this experiment. The birds were kept in individual cages and fed ad lib a geese commercial raising mash containing 15% protein and 2800 calories per kg. They were subjected to 14 hours light daily.

Surgical Procedure

The geese were anesthetized via the brachial vein with Brietal Sodium (Metoxton Sodium, Eli Lilly). The heads were fixed in an avian type stereotaxic instrument, specifically adapted for geese. Preliminary studies of the sagittal roentgenograms of the goose's skull were carried out, relating skull structures to brain anatomy so that it was possible to locate hypothalamic and other brain areas. This was done exploiting the procedure for placing brain lesions or implanting cannulas previously described in the x-ray atlas of the chicken diencephalon [31]. The position of the

head was maintained at 45° to eliminate damage in frontal striatal centers, while penetrating the brain with the cannulas. Such damage was found to cause motor disturbances (Authors' unpublished data).

In order to produce brain damage, 200 μg of 6-OHDA together with 40 μg vit. C dissolved in 5 μl saline were stereotaxically injected through a 27 gauge syringe needle. This was based on preliminary studies made to establish the correct dosage of 6-OHDA. The following dosages were used in these experiments: 25, 50, 75, 100, 150, 200 and 250 μg. A high mortality rate resulted when 250 μg 6-OHDA was given but when 150 μg or less was given there were no demonstrable effects (i.e. hyperhagia, obesity, etc.). Therefore, the 200 μg dosage was selected. In various groups of geese the following brain areas were destroyed: the VMH bilaterally or unilaterally; the bilateral hypothalamic areas located dorsal, lateral, caudal and rostral to the VMH, excluding the lateral hypothalamic area, and the bilateral septal area.

Parameters Measured and Autopsy Procedure

After surgery the birds were returned to their cages. Daily food intake and periodic body weights were measured. Thirty-eight days after surgery the geese were sacrificed by decaptiation. The brains were immediately removed and fixed in Heidenhain's mercury chloride-free solution for 24 hr and transferred to 10% formolsaline. Serial frontal frozen sections of 30 μm were prepared and stained with cresylecht-violet and examined microscopically for localization of brain damage.

The following organs were removed, cleaned from adhering tissues and weighed: abdominal adipose tissue, liver, gonads, pituitary, adrenal and thyroid glands. Liver samples were fixed in 10% neutral buffered Formalin, paraffin sections of 5 μm were prepared and stained with Hematoxylin-Eosin. Fresh samples of liver and abdominal adipose tissue were taken for determination of fat content [19]. Statistical analysis of the data was carried out using a computer program for analysis of variance and Multiple Range Test.

RESULTS

Roentgenogram of the Goose Skull

Figure 1 presents a typical sagittal x-ray picture of the goose skull, taken while the head was secured in the stereotaxic instrument. In this case the needle tip was located in the VMH. Clear bony structures can be visualized and may serve as landmarks enabling the operator to reach the desired brain areas by stereotaxic manipulation [31].

Brain Histology

Figure 2 shows representative schematical drawings of frontal sections of brains fron the bilateral VMH (BL-VMH), unilateral VMH(UL-VMH) and septal area injected birds. Only the center of the damage is depicted. In some cases, the bilateral VMH damage was extended in a more posterior direction destroying the ventrorostral part of the mamillary nuclei. Brain damage was also identified in all other hypothalamic injection sites mentioned. The damage caused by the 6-OHDA injection seemed to be nonspecific. It destroyed both cell bodies and axons, recalling the histological appearance of the brain damage caused by an electrolytic lesion.

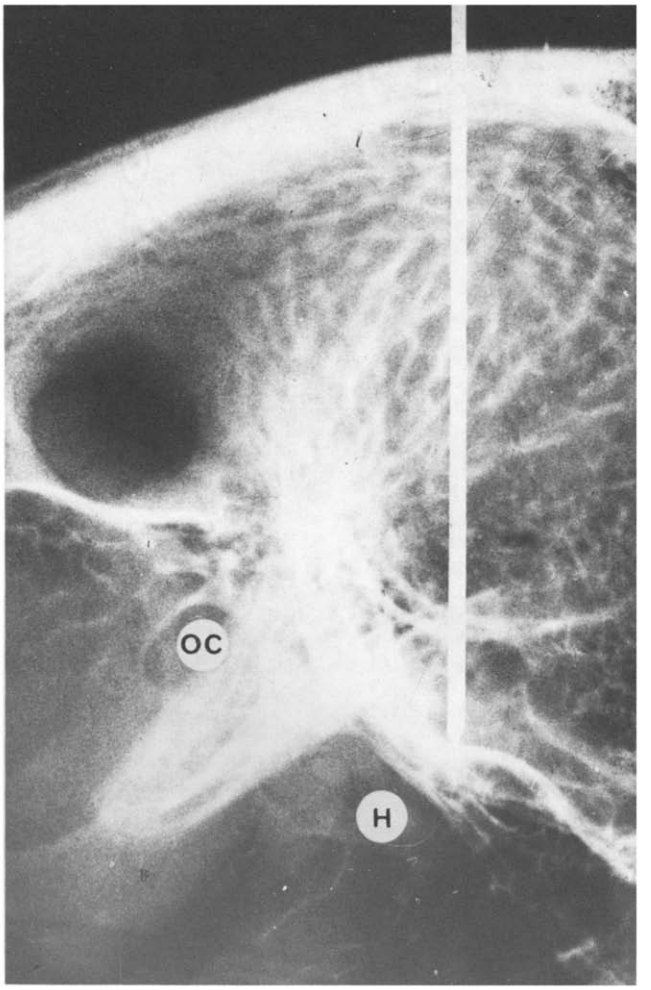

FIG. 1. Sagittal roentgenogram of a goose head, with a needle inserted into the VMH area. OC = optic chiasma; H = hypophysis (x6).

Food Intake and Body Weight

Figures 3 and 4 respectively show the average daily food intake and body weight of the various groups of geese participating. The BL-VMH lesioned geese demonstrated the highest food intake which started soon after surgery (Fig. 3). Their food intake decreased gradually from the 20th day post surgery, returning to a normal level around the 30th day. An increased food intake was also shown by the UL-VMH and septal area lesioned geese over controls, although to a markedly lesser extent. However, while the septal lesioned birds remained hyperphagic until the end of the experimental period, a definite gradual decrease in food intake was shown by the UL-VMH lesioned birds. All other hypothalamic lesioned geese showed no differences from the controls in their food intake.

A significant increase in body weight was found only in the BL-VMH lesioned geese (Fig. 4). As the experiment progressed, the septal lesioned geese demonstrated a pattern of body weight lower than that of the controls.

Autopsy

Results, including fat determination are presented in

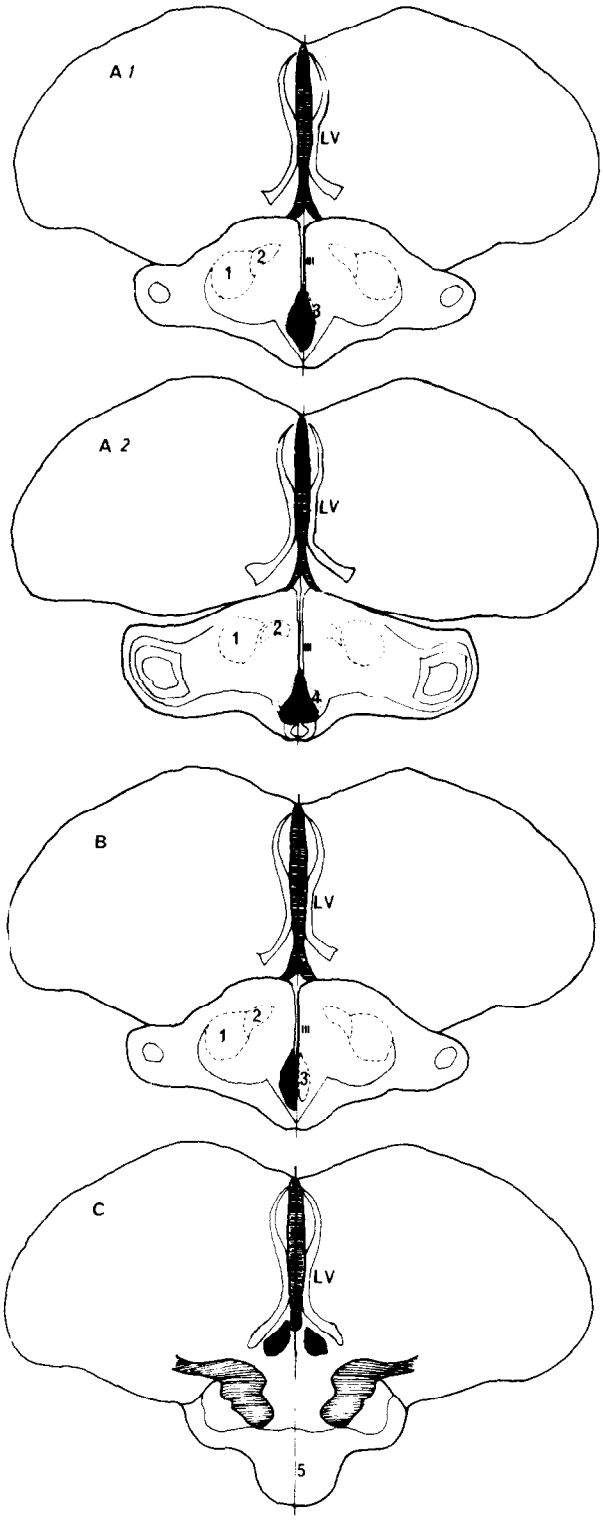

FIG.2. Schematical drawings of frontal brain sections of representative geese, showing the location of the lesions caused by injection of 6-OHDA into the bilateral VMH-mammilary area (A_1 and A_2), unilateral VMH (B) and bilateral septal area (C). The drawings depict the center of the lesion. The plane of the sections was at a right angle position to an imaginary line connecting the tops of the cerebral hemisphere and the cerebellum. LV = Lateral Ventricles; III = Third Ventricle; 1 = Rotundus Nucleus; 2 = Ovoid Nucleus; 3 = VMH; 4 = Mammilary Nucleus; 5 = Optic Chiasma.

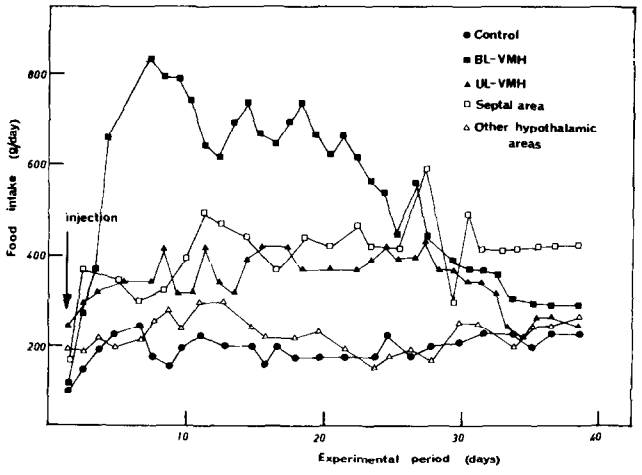

FIG. 3. Average daily food intake of the lesioned and control groups of geese. The average S. E. for the five groups of geese, calculated for the entire experimental period is 24.1.

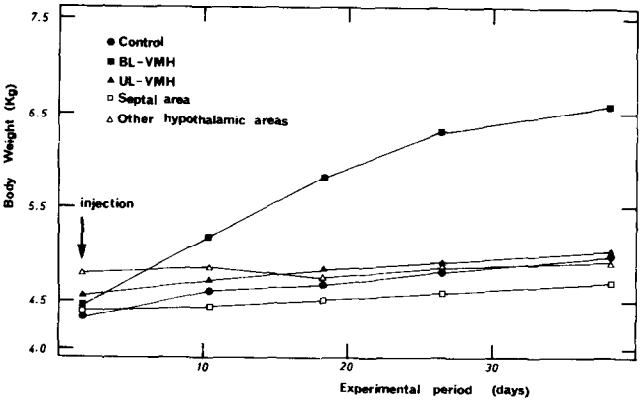

FIG. 4. Average periodical body weight of the lesioned and control groups of geese. The average S. E. for the five groups of geese, calculated for the entire experimental period is 61.0.

Table 1. The weight of abdominal adipose tissue of the BL-VMH lesioned geese was significantly higher than that of all other groups which showed no significant differences among them. However, a clear tendency of a lower abdominal adipose tissue weight can be seen in the septal area lesioned geese. Similar percentages of fat in abdominal adipose tissue were obtained in all experimental groups.

Average liver weight of the BL-VMH lesioned geese was three times as large as that of the others all of which had similar weights. Total liver fat was four times greater in the BL-VMH lesioned group than found in the controls or in the geese lesioned in the hypothalamic areas, other than the VMH. Higher levels of liver fat were found in the septal area and in the UL-VMH lesioned geese when compared to the controls.

There were no significant differences among the various groups of geese in respect to the weights of the pituitary, adrenal and thyroid glands. A significant decrease in testes weight was obtained in the BL-VMH lesioned birds as compared to the controls.

Figure 5 shows pictures of representative histological

TABLE 1

DIFFERENCE BETWEEN FINAL AND INITIAL BODY WEIGHT, WEIGHT OF SELECTED ORGANS AND GLANDS, AND FAT CONTENT OF LIVER AND ADIPOSE TISSUE IN THE CONTROL AND LESIONED GROUPS OF GEESE (MEANS ± SE)

Treatment	n	Difference in B.W. (g)	Abd. adipose weight (g)	Liver weight (g)	Abd. adipose tissue fat content (%)	Liver fat content (%)	Testes weight (g)	Pituitary weight (mg)	Thyroid weight (mg)	Adrenal weight (mg)
Control	9	370 ± 134‡*	192 ± 14‡	94 ± 4‡	85.3 ± 3.8†	13.0 ± 2.0§	3.05 ± 0.37†	22.8 ± 3.7†	323 ± 24†	619 ± 44†
Lesioned Geese										
Bilateral VMH	23	1390 ± 124†	394 ± 24†	277 ± 21†	89.2 ± 1.6†	49.0 ± 4.8†	0.40 ± 0.10§	22.0 ± 1.2†	337 ± 30†	670 ± 33†
Unilateral VMH	6	390 ± 210‡	206 ± 38‡	99 ± 18‡	92.3 ± 1.3†	21.9 ± 5.9‡	1.41 ± 0.15†‡	27.8 ± 5.6†	319 ± 28†	562 ± 34†
Septal area	6	236 ± 110‡	153 ± 24‡	84 ± 13‡	86.9 ± 5.6†	21.4 ± 4.5‡	0.93 ± 0.54†‡§	25.1 ± 2.6†	236 ± 24†	521 ± 28†
Other hypoth. area	29	170 ± 82‡	174 ± 15‡	84 ± 6‡	85.8 ± 3.7†	11.7 ± 0.7§	1.38 ± 0.33†‡	30.9 ± 2.7†	297 ± 17†	590 ± 25†

*Figures which are not marked by the same symbol are statistically different from each other ($p<0.05$-0.01).

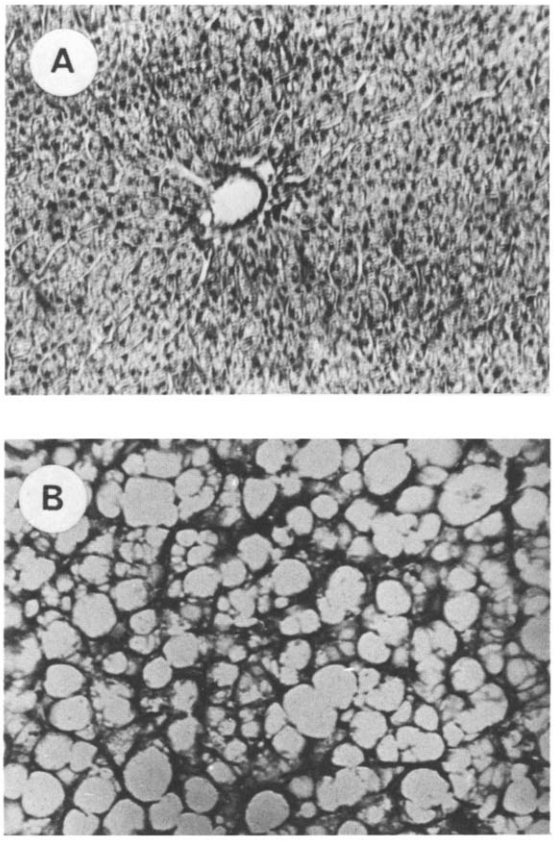

FIG. 5. Pictures of representative liver histological sections of a control goose (A) and of a BL-VMH lesioned one (B). Hematoxylin Eosin (X 280).

sections of the liver of a BL-VMH lesioned goose and of a control. Large vacuoles containing fat appear in the liver cells of the lesioned bird.

DISCUSSION

The brain damage caused by intracranial injections of 6-OHDA is considered by some workers as specific, destroying adrenergic and dopaminergic nerve terminals [9, 10, 11, 32,]. The results of this investigation support the concept of Poirier *et al.* [22] who demonstrated the nonspecific brain damage caused by 6-OHDA.

Our results with BL-VMH lesioned geese agree with Auffray and Blum [2] and Auffray *et al.* [1] in respect to the hyperphagia developed in BL- VMH lesioned geese. In contrast to the domestic fowl, where the hyperphagia starts to develop only 5–7 days post lesioning [28], the goose responds much more quickly.

When employing the force-feeding techinque commonly used for production of goose fatty liver, only small amounts of food can be force-fed during the first and second week (250–500 g/day) [20]. However, greater amounts of food were voluntarily consumed by the BL-VMH lesioned geese during the first 14 days post surgery.

It may be suggested that in BL-VMH lesioned geese there is an immediate change in the digestibility and rate of movement of food in the intestinal tract, a subject which needs further investigation. Lepkovsky [15] suggested that the VMH lesion causes a primary change in adipose tissue by raising its set point, thus inducing a compensatory increase in the rate of food transport and its digestibility in the intestinal tract. These physiological changes elicit hyperphagia, which probably develops as a secondary reaction. The marked obesity note in the BL-VMH lesioned geese substantiates the concept of a change of the set point in the adipose tissue.

In contrast to the rat, in which UL-VMH lesion causes moderate obesity [17] no such phenomenon was obtained in the domestic fowl (Authors' unpublished data), and in the goose as found in the present work. Investigations with the fowl showed no development of hyperphagia due to UL-VMH destruction. Perhaps the transient increase in food intake, exhibited in the UL-VMH lesioned geese in the present work, may be related to the temporary pressure produced by oedema which developed in the contralateral VMH area as a result of the surgical procedure. The lack of effect of hypothalamic lesions, other than that of the VMH area, on food intake and body weight in the goose, is in agreement with the results obtained in the domestic fowl [6].

Moderate but constant hyperphagia unaccompanied by obesity was demonstrated in the septal lesioned geese. This phenomenon was observed also in the rat [5, 13, 26] and domestic fowl (Robinzon *et al.*, unpublished data). In the latter, long lasting increase in food intake without obesity

due to septal lesions, may be partly explained on the basis of an increase in thyroid activity, indicated by both metabolic and histological criteria.

Figures 6 and 7 respectively show graphical scattering of the differences between the final and the initial body weight (△BW) in relation to the average daily food intake, and of the △BW in relation to the weight of adipose tissue. It is evident that a positive correlation exists between the △BW and the average food intake (Fig. 6) and that the increase in body weight was attained mainly because of body fat (Fig. 7).

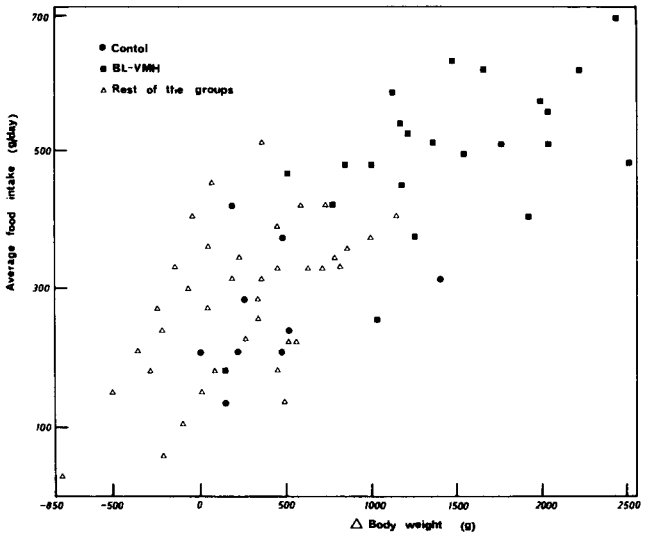

FIG. 6. Graphical scattering of the relationship between △BW and average daily food intake of the individual geese r = 0.737, $p < 0.001$.

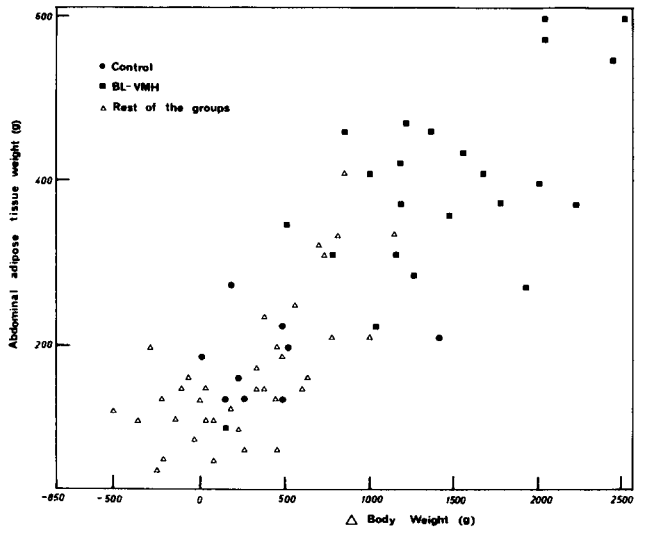

FIG. 7. Graphical scattering of the relationship between △BW and weight of abdominal adipose tissue of the individual geese r = 0.723, $p<0.001$.

In principle the increase in the fat content of the liver which occurred in the hyperphagic BL-VMH lesioned geese parallels artificial force-feeding of geese [20]. However, the absolute weights of the liver noted in this lesioned group of geese was considerably lower than that commonly obtained in the force-fed ones. The Landes breed of geese used by Auffray and Blum [2] is much heavier than the one used in the present investigation. This can partly explain the greater weight of the fatty livers obtained by those investigators.

Since the liver serves as the main source of lipogenesis in avian species [7,21], a moderate increase in food intake may cause an increase in liver fat content. This possibly was the case in the UL-VMH and septal area lesioned geese.

Whether the steatosis of the liver in those geese with the hypothalamic lesions remains steady or is subjected to changes affected by the different phases of food intake of the hypothalamic obesity, needs further clarification.

The relationship between liver weight and abdominal adipose tissue weight and between liver weight and daily food intake, are demonstrated by the graphical scattering presented in Figs. 8 and 9 respectively.

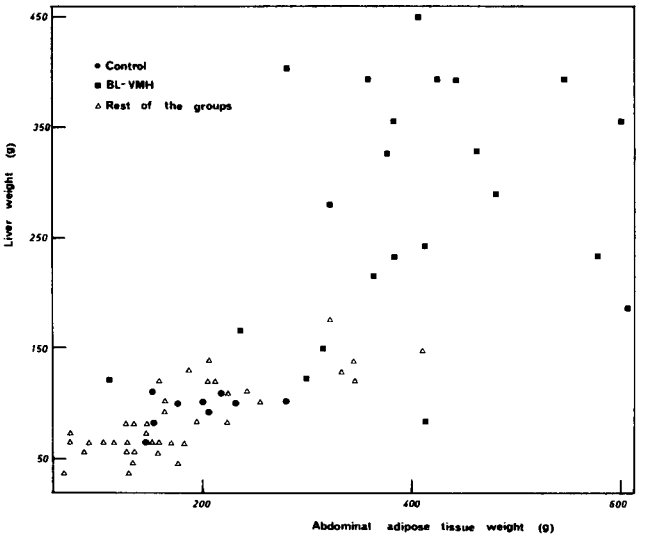

FIG. 8. Graphical scattering of the relationship between liver weight and abdominal adipose tissue weight of the individual geese r = 0.770, $p<0.001$.

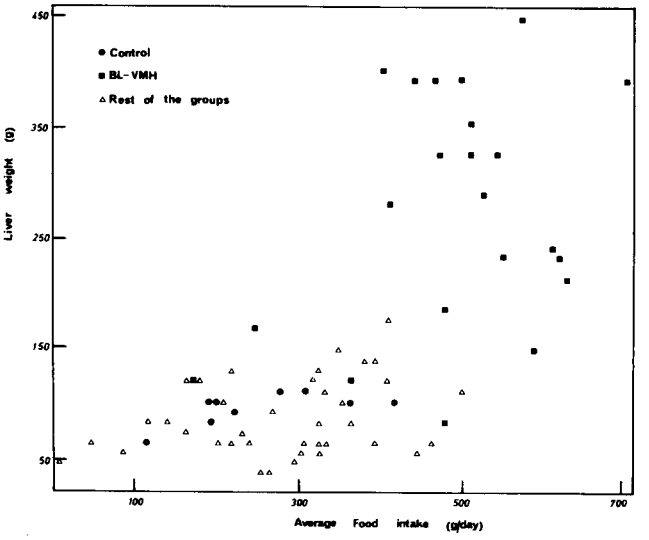

FIG. 9. Graphical scattering of the relationship between liver weight and average daily food intake of the individual geese r = 0.745, $p<0.001$.

The following may be concluded: (1) A clear pattern of a positive correlation exists between liver and abdominal adipose tissue weights in all groups of geese. However in the BL-VMH lesioned geese there is no correlation($r = 0.075$ $p>0.1$); (2) A correlative pattern could be found between liver weight and average daily food intake except for the BL-VMH lesioned geese ($r = 0.124$ $p>0.1$); and (3) The liver weights of the BL-VMH lesioned geese, which were higher than the others, also showed greater individual variations. Food intake varied less in relation to liver weight than abdominal adipose tissue weight.

In contrast to the BL-VMH lesioned geese, where no significant correlation was found between weight and food intake, a significant positive correlation coefficient was obtained between these parameters in the case of the artificially force-fed geese [20]. This discrepancy is likely to be a consequence of differences in the origin of the obesity in the two categories of over-fed geese. The artificially force-fed animals receive the food in excess of their bodily needs, but those with the hypothalamic lesions eat voluntarily to satisfy their new set point. A significant correlation between liver weight and its total fat content was found in BL-VMH lesioned geese ($r = 0.870$, $p<0.01$) and in artifically force-fed geese [20].

The marked decline in testes weight of the BL-VMH lesioned geese is in accordance with the same findings in the domestic fowl, lesioned in the basomedial hypothalamic area [23]. Since the testes of geese at this age are not fully matured, the results should be considered as arrestment rather than atrophy. The other groups of geese also showed moderate decrease in testes weight. This can be explained on the basis of interference of the brain lesions with the neuroendocrine-gonadal axis. No inverse relationship between testes weight and body fat was found in the BL-VMH lesioned geese, at this age. On the other hand, a significant negative correlation between these parameters, was found in mature White Leghorn cocks, bearing the same kind of lesions [28].

ACKNOWLEDGEMENT

The authors wish to thank the U.S.-Israel Binational Science foundation (Grant No. 189) and Mr. J. Taylor, London, England for supporting this investigation.

REFERENCES

1. Auffray, P., J. C. Marcilloux, C. Bahy and D. Albe-Fessard. Hyperphagie induite chez l'oie par injections intraventriculaires du 6-hydroxydopamine. *C. R. Acad. Sci. Ser. D-Sci. Natur.* **276:** 347–350, 1973.
2. Auffray, P., and J. C. Blum. Hyperphagie et steatose hepatique chez l'oie apres lesion du noyau ventro median de l'hypothalamus. *C. R. Acad. Sci. Paris, Ser. D-Sci. Natur.* **270:** 2362–2365, 1970.
3. Baile, C. A., J. Mayer, A. W. Mahoney and C. McLaughin. Hypothalamic hyperphagia in goats and some observations of its effect on glucose utilization ratio. *J. Dairy Sci.* **52:** 101–109, 1969.
4. Brobeck, J. R. Mechanism of the development of obesity in animals with hypothalamic lesions. *Physiol. Rev.* **26:** 541–559, 1946.
5. Donovick, P. J., R. G. Burright and P. L. Gittelson. Body weight and food and water consumption in septal lesioned and operated control rats. *Psychol. Rep.* **25:** 303–310, 1969.
6. Feldman, S. E., N. Snapir, M. Yasuda, F. Treuting and S. Lepkovsky. Physiological and nutritional consequences of brain lesions: A functional atlas of the chicken hypothalamus. *Hilgardia* **41:** 605–629, 1973.
7. Goodridge, H. G. and E. G. Ball. Lipogenesis in the pigeon: in vivo studies. *Am. J. Physiol.* **213:** 245–249, 1967.
8. Hamilton, C. L. An observation of long-term experimental obesity and diabetes mellitus in the monkey. *J. Med. Prim.* **1:**247–255, 1972.
9. Heikkila, R. E. and G. Cohen. Inhibition of biogenic amine uptake by hydrogen peroxide: A mechanism for toxic effect of 6-OHDA. *Science* **172:** 1257–1258, 1971.
10. Heikkila, R. E. and G. Cohen. In vivo generation of hydrogen peroxide from 6-OHDA. *Experientia* **28:** 1197–1198, 1972.
11. Kaplanski, J. and P. G. Smelik. Pituitary-adrenal activity and depletion of brain catechol amines after central administration of 6-OHDA. *Res. Communs chem. Pathol. Pharmac.* **5:** 263–271, 1973.
12. Khalaf, F. Hyperphagia and aphagia in swine with induced hypothalamic lesions *Res. vet. Sci.* **10:** 514–517, 1969.
13. Kondo, C. Y. and S. A. Lorens. Sex differences in the effects of septal lesions. *Physiol. Behav.* **6:** 481–485, 1971.
14. Kuenzel,W. J. and C. W. Helms. Hyperphagia, polydipsia and other effects of hypothalamic lesions in the white- throated sparrow, Zonotrichia-albicollis. *Condor* **72:** 66–75, 1970.
15. Lepkovsky, S. Newer concipts in the regulation of food intake. *Am. J. clin. Nutr.* **26:** 271–284, 1973.
16. Lepkovsky, S. and M. Yasuda. Hypothalamic lesions, growth and body composition of male chicken. *Poultry Sci.* **45:** 582–588, 1966.
17. Marshall, J. F. Increased orientation to sensory stimuli following medial hypothalamic damage in rats. *Brain Res.* **86:** 373–387, 1975.
18. Mayer, J. Genetic, traumatic and environmental factors in the etiology of obesity. *Phsiol. Rev.* **33:** 472–508, 1953.
19. Nir, I. and M. Perek. The effect of various protein levels in feed of goslings during the preparatory period on fatty liver production and blood plasma components. *Ann. Biol. anim. Biochem. Biophys.* **11:** 645–656, 1971.
20. Nir, I., M. Perek and Z. Katz. The influence of soybean meal supplemented to the maize diet of forced-fed geese upon their liver, organ and blood plasma components. *Ann. Biol. anim. Biochem. Biophys.* **12:** 77–89, 1972.
21. O'Hea, E. K. and G. A. Leveille. Lipid biosynthesis and transport in the domestic chick (Gallus Domesticus). *Comp. Biochem. Physiol.* **30:** 149–159, 1969.
22. Poirier, L. J., R. Langelier, A. Boberge, R. Boucher and A. Kitsikis. Non-specific histopathological changes induced by the intracerebral injection of 6-Hydroxydopamine (6-OHDA). *J. meurol. Sci.* **16:** 401–416, 1972.
23. Ravona, H., N. Snapir and M. Perek. The effect of electrolytic lesions in various lacations of the basal hypothalamus on the gonadal axis in cockerels. *Gen. comp. Endocr.* **20:** 112–124, 1973.
24. Ravona, H., N. Snapir and M. Perek. Histological changes of the pituitary gland in cockerels bearing basal hypothalamic lesions and demonstration of its anti-HCG immunofluorescence reacting cells. *Gen. comp. Endocr.* **20:** 490–497, 1973.
25. Russek, M. and P. J. Morgane. Aborexic effect of intraperitoneal glucose in the hypothalamic hyperphagic cat. *Nature* **199:** 1004–1005, 1963.
26. Singh, D. and D. Meyer. Eating and drinking by rats with lesions of the septum and the ventromedial hypothalamus. *J. comp. physiol. Psychol.* **65:** 163–166, 1968.
27. Snapir, N., I. Nir, F. Furuta and S. Lepkovsky. Effect of administered testosterone propionate on cocks functionally castrated by hypothalamic lesions. *Endocrinology* **84:** 611–617, 1969,

28. Snapir, N., H. Ravona and M. Perek. Effect of electrolytic lesions in various regions of the basal hypothalamus in White Leghorn cockerels upon food intake, obesity, blood plasma triglycerides and protein. *Poultry Sci.* **52:** 629–636, 1973.
29. Snapir, N., I. Nir, F. Furuta and S. Lepkovsky. Effects of functional and surgical castration of White Leghorn cockerels and replacement therapy on food intake, obesity, reproductive traits and certain components of blood, liver, muscle and bone. *Gen. comp. Endocr.* **24:** 53–64, 1974.
30. Snapir, N., S. Lepkovsky, H. Ravona and M. Perek. Plasma testosterone in functionally castrated cockerels with hypothalamic lesions. *Br. Poult. Sci.* **15:** 441–448, 1974.
31. Snapir, N., I. M. Sharon, F. Furuta, S. E. Feldman, S. Lepkovsky, H. Ravona, and B. Robinzon. An X-ray atlas of the sagittal plane of the chicken diencephalon and its use in the precise localization of brain sites. *Physiol. Behav.* **12:** 419–424, 1974.
32. Stein, C., P. C. Wise and B. D. Berger. Noradrenergic reward mechanism, recovery of function and schizoprenia. In: *The Chemistry of Mood Motivation and Memory*, edited by J. L. McGaugh. New York: Plenum Publishing Corporation, pp. 81–103, 1973.