EXPERIMENTAL OBESITY IN THE DOG

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Received for publication February 4, 1944

Experimenters in the field of obesity are aware of the controversy which still exists concerning the relative importance of lesions of the hypophysis and of the hypothalamus as causes of experimental obesity. Fröhlich (1) and Crowe, Cushing and Humans (2) are among those who attributed to lesions of the hypophysis a dominant rôle. Aschner (3), Erdheim (4), Bailey and Bremer (5), Camus and Roussy (6), Smith (7), Grafe and Grünthal (8), Economo (9) and others have declared that lesions of the hypothalamus alone may give rise to obesity. In the opinion of the authors, analysis of the literature yields evidence which supports the interpretation that either a lesion of the hypophysis or of the hypothalamus or both together may lead to obesity. Little or no explanation as to how such lesions may cause obesity has been offered.

In this paper observations on the effect of removal of the hypophysis and of various lesions of the hypothalamus on fat storage in the dog are presented. It has been found that either a removal of the hypophysis or the production of a lesion of the hypothalamus resulting in partial or complete destruction or denervation of the paired paraventricular nuclei may cause obesity. The additional bilateral destruction or denervation of the supraoptic nucleus enhances the rate of development of the obesity. The maximum degree of obesity follows a properly placed hypothalamic lesion without removal of the hypophysis. Following such a hypothalamic lesion there occur a loss and degeneration of the basophil cells of the hypophysis. The adrenal cortex does not atrophy; indeed, the animal becomes in a manner sensitized to this hormone, thereby favoring gluconeogenesis. This is believed to be significant in the pathogenesis of obesity through an effect on hunger and on fat formation and storage. The depression of thyroid and gonadic function which follows removal of the hypophysis or degeneration of its basophil cells also in itself leads to obesity at a slow rate, particularly when the dogs so modified are caged and given an ample food supply. A rapid gain in weight is invariably accompanied by a large food intake. It is concluded that the development of obesity is an expression of an excess of food intake over food requirements and not of any abnormal metabolic processes. With carbohydrate available, fat formation and storage are facilitated.

Materials and Methods. Mongrel dogs were used as experimental animals. Most of them were kept in metabolism cages from one to three months to establish their weight and to determine their normal daily urine output. They were fed measured limited amounts of dog chow and horse meat except when for

1 Recipients of a grant-in-aid of research from the Commonwealth Fund.

2 On leave of absence for military duty.
experimental purposes unlimited but measured quantities were allowed. A few normal dogs were kept in cages for 6 months to 2 years under the standard conditions noted to serve as controls, while the remainder were subjected to various operative procedures. At least 10 to 20 animals of each of the different groups, proven so by thorough examination of serially cut microscopic sections of essential tissues were available for study. In this paper, to conserve space, the results on only a few of each class will be presented. The results on the others of the classes conformed with those reported on.

The required operations on the hypophysis and the hypothalamus were carried out through the oral approach under nembutal anesthesia and designed so as to yield the following groups:

1. Simple hypophysectomy dogs; those in which the entire glandular hypophysis, the stalk and the posterior lobe of the neural hypophysis were removed. The median eminence was not appreciably, if at all, injured.

2. Total hypophysectomy dogs; those in which the entire glandular and neural divisions of the hypophysis were removed, i.e., the median eminence was included.

3. Puncture dogs; these were subdivided into three groups. A. Those in which the stalk was severed, care being taken to disturb the hypophysis as little as possible, and then with a small scoop the median eminence was removed and a puncture wound made into the posterior hypothalamus to sever the fibers passing caudally from the paired paraventricular nuclei.

B. Those in which after severing the stalk and removing the median eminence the puncture wound was made rostrally in the central portion of the anterior hypothalamus.

C. Those in which after severing the stalk a puncture wound only was made in the mid-posterior hypothalamus deep enough to interrupt the fibers coming from the caudal portion of the paired paraventricular nuclei.

The dogs in the various classes were then observed for periods varying from 5 to 30 months. They were kept under dietary conditions similar to those used before operation. Periodically, both before and after operation, unlimited amounts of dog chow and meat were allowed for a period of a week and the amount of food eaten measured. It was hoped that the amounts consumed before and after operation would serve as a measure of the dog's hunger or state of satiation. Daily urine measurements were made and weekly records kept. Periodically the blood glucose and the blood cholesterol levels were determined. Insulin and sugar tolerance determinations also were made. After the period of observation the dogs were sacrificed and completely autopsied. On a limited number of dogs from each of the classes liver glycogen and liver fatty acid determinations were made in the postabsorptive stage (16 to 24 hrs. after a last meal) at the time of sacrifice.

At autopsy the brain was perfused in situ with 10 per cent formalin and then fixed in this solution for several days. Then the hypothalamus was removed, serially sectioned at 20 microns and every third slide stained with cresyl violet. The sella and its contents were similarly sectioned at 20 microns and stained with
hematoxylin and eosin. The sellar contents from many of the dogs of group 3 were separated carefully from the bone after fixation to eliminate the necessity of using decalcifying agents and to permit staining of the hypophysial tissue for differentiation of cell types according to the method of Rasmussen. Such tissue was serially sectioned at 5 microns.

Among the other organs and tissues studied for this research were the thyroid, the pancreas, the liver, the adrenals and the gonads. Care was taken to obtain sections from 5 to 7 different areas of the pancreas. These were stained with hematoxylin and eosin and according to the method of Masson and the Mallory-azan technique.

**RESULTS.** Analysis of the body weight and urine charts. Normal dogs kept under the conditions outlined above vary in their response as regards body weight. Certain breeds which are known to have a tendency to gain weight with age may in 2 years of observation exhibit a definite but slowly developing weight gain of from 30 to 65 per cent, while other types may show only a slight weight increase from 0 to 10 per cent (fig. 1). It has been learned from our experiences in attempting to establish a base line for normal dogs for comparison with dogs modified by operative procedures that ideally, pure bred dogs, of a breed which does not normally exhibit a definite tendency to obesity, should be chosen for an investigation of experimental obesity.
With increasing obesity normal dogs exhibit no alteration in urine output (fig. 2).

Simple hypophysectomy dogs exhibit a definite tendency to obesity. The development of their obesity is slow but definitely more rapid than that which occurs in the normal dog with spontaneously developing obesity. Such dogs may exhibit an increase of 30 to 85 per cent over their original weight after 2 years of observation (fig. 1). The weight curves of simple hypophysectomy dogs indicate that conclusions as to the effect of the procedure on obesity must not be drawn before an adequate period of time has elapsed. In healthy animals after long periods of observation, i.e., 1½ to 2 years or more, the degree of weight increase is from 20 to 30 per cent greater in simple hypophysectomy dogs than in normal dogs with a definite tendency to obesity.

The urine output of this class of dogs is normal, or normal with an occasional slight increase over the normal (fig. 2).

Total hypophysectomy dogs exhibit a much more rapidly developing obesity. The degree of weight increase in the first year after operation is usually definitely greater than that of the simple hypophysectomy dog but after 2 or more years of observation the 2 classes exhibit very similar weight increases (fig. 1).

Total hypophysectomy dogs exhibit a permanent and marked diabetes insipidus.

Puncture dogs of the group A class exhibit the most rapidly developing and
the greatest degree of experimental obesity. After 2 or more years of observation the weight increase may reach 150 per cent above the preoperative level (fig. 1). The degree of increase is greater than that exhibited by the other classes of dogs. Such dogs are modified by the operation in a manner which results during the period of rapidly developing obesity in a greater food intake than in any of the other classes of dogs.

Puncture dogs exhibit a permanent maximum diabetes insipidus (fig. 2).

Group B dogs exhibit only a slowly developing moderate degree of obesity. It has not been possible to produce a lesion which leads to a state of marked diabetes insipidus without the development of any obesity. From the clinical literature it is evident that in man such a condition is possible. It is felt that in the dog complete bilateral destruction of the supra-optic nucleus alone would effect such a result but this can not be accomplished by our method of operation. Always in our dogs with destruction or denervation of the supraoptic nuclei the median eminence is removed and this is associated with a mechanical loss or a retrograde degeneration of part of the cells of the rostral portion of the paraventricular nuclei.

Group C dogs exhibit a rapid, marked increase in weight. The amount of weight increase is slightly less in our experience than when the entire supra-optico-hypophysial system is destroyed. From an analysis of our data the impression is gained that complete or marked loss of pitressin aids in the development of obesity. It is realized that the degree of difference in weight increase between dogs of the A and C groups of this class of dogs might have been found to be within the range of variation for those of group A if the number of dogs operated on had been larger. There exists, however, a possible anatomical basis for a difference between these two groups of dogs. Thus the destruction or denervation of the paired paraventricular nuclei which results in obesity is partially effected in total hypophysectomy dogs because, as before stated, destruction of the median eminence results in a marked loss of cells from the rostral portion of this nucleus. This cell loss conceivably may be of significance in effecting those changes in the body which lead to the obesity which is evident in greater degree on destruction of the major portion of the entire paraventricular nucleus through a posterior hypothalamic puncture.

Anatomical studies. Microscopic examination of the hypophysis and the hypothalamus from 2 normal dogs which became markedly obese showed both to be cytologically normal (fig. 3). Microscopic examination of the adrenals, the gonads, the liver and the pancreas reveals nothing obviously abnormal. The thyroid gland of one showed low acinar epithelium with inspissated colloid, that of the other showed large but vacuolated cells without colloid formation. Such a finding has been reported for the hibernating hedgehog by Peiser (10). The distribution of the accumulated fat was similar to that found in dogs which became obese after the operative procedures.

Examination of the hypothalamus after simple hypophysectomy reveals an 80 to 85 per cent loss of the cells of the supraoptic nuclei. The paraventricular nuclei are practically unaffected (fig. 4).
Total hypophysectomy, i.e., median eminence included, results in a complete loss of the cells of the supraoptic nucleus and a 30 to 50 per cent loss of the cells of the rostral portion of the paraventricular nucleus. No other nuclei or hypothalamic areas are appreciably affected (fig. 5).

Simple and total hypophysectomy results in changes in other tissues and endocrine glands which are of significance in our problem (figs. 4 and 5). The fat storage which results occurs in the subcutaneous tissue of the neck, the trunk, the abdominal wall and the extremities. There is an increase of fat in the pericardium, in the omentum, in the retroperitoneal areas, particularly in the perirenal regions. There is no obvious structural difference in the fatty tissues grossly or microscopically, from similar fatty tissues of the normal dog.

To determine the hypothalamic lesion responsible for fat storage lesions were made in more than 200 dogs. At various intervals after the operation the dogs were sacrificed and the hypothalami serially sectioned and stained for cells with cresyl violet. It can be stated from a correlation between the histological lesion and the effect on body weight that lesions of the anterior hypothalamus alone do not cause obesity. Lesions of the posterior hypophyseal nuclei do. From observations such as are depicted in figure 6 on dogs 9 and 10 it was suspected that the bilateral destruction or degeneration of the paraventricular nucleus was the essential lesion.

In each of these 2 dogs a lesion, caudal to the region of the paraventricular nuclei was made in the posterior hypothalamus. The pars distalis and posterior lobe also were removed in both, but the median eminence was not disturbed appreciably. These 2 dogs were chosen because of a critical difference in their

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Fig. 3. Photomicrographs of tissues from dog 2, a normal dog which became obese. A. supraoptic nucleus × 45; B. paraventricular nucleus × 45; C. thyroid gland × 610. Note the absence of colloid, cytoplasm vesicular. D. pancreas × 45; E. adrenal × 45; F. ovary × 45.

Fig. 4. Photomicrographs of tissues from simple hypophysectomy dog 4. A, supraoptic nucleus × 45; at least 20 per cent of the cells remain. B, paraventricular nucleus × 45, caudal portion. C, thyroid gland × 45. Note small acinar, inspissated colloid, many acini without colloid. D, pancreas × 45 showing hyperplasia and hypertrophy of islets. E, adrenal × 45 showing marked atrophy of the zona fasciculata and of the zona reticularis. The zona glomerulosa is of normal width but the nuclei are pale staining, the cytoplasm vesicular. The medullary tissue also is atrophied. F, ovary × 45. Note the atretic follicles, absence of mature follicles.

Fig. 5. Photomicrographs of tissues from total hypophysectomy dog 5. A, supraoptic nucleus × 45. Note absence of cells. B, paraventricular nucleus × 45, caudal portion; at least 80 to 85 per cent of the cells present. C, thyroid × 45. Note flat acinar epithelium, many acini without colloid. D, pancreas × 45. E, adrenal × 45. Note marked atrophy of all zones except the glomerular. The cells in this show pale staining nuclei, the cytoplasm is vesicular and the medulla is atrophied. F, ovary × 45. Note only immature follicles.

Fig. 9. Photomicrographs of tissues from dog 8, of the puncture type. A, supraoptic nucleus × 22. Note absence of cells. B, paraventricular nucleus × 22, caudal portion. Note absence of cells. C, thyroid × 22. D, pancreas × 22. The islets are small, the cells show degenerative changes. E, adrenal × 22. Note the normality of the zones. F, ovary × 22. Note absence of mature follicles.
lesions. In one, which became obese, the cells in the caudal portions of the paraventricular nuclei are absent (retrograde degeneration); in the animal which did not become obese the nucleus has its normal length (fig. 7). The shortening of the paraventricular nuclei in the obese dog is due to retrograde degeneration of the cells following the interruption of their axons by the posterior hypothalamic puncture. The puncture in the other dog did not sever these axons. The paraventricular nuclei give rise to fibers going to the neural divisions of the hypophysis and to fibers which descend down the brain stem.

After this observation all our material was rechecked and a definite correlation between the degree of obesity and the degree of bilateral destruction or cell loss of the paraventricular nuclei established.

![Graphs showing percentage increase in weight and urine output](http://ajplegacy.physiology.org/)

**Fig. 6.** Plots of percentage increase in weight against time and of urine output in days after operation for dogs 9 and 10.

The cell loss of most significance in causing obesity is from the caudal portion of the nucleus and must be bilateral. The rostral portion of the paraventricular nuclei innervates principally the median eminence and these cells probably innervate mostly or only pituicytes (Heinbecker and White, 11). The maximum degree of obesity results when the entire paraventricular nucleus disappears, partial loss results in only moderate obesity. Bilateral destruction of the lateral mammillary and of the mammillary nuclei does not lead to obesity.

Support for the above conclusion also has been found in the histological studies of the hypothalami of 4 cases of Cushing's syndrome in all of which obesity was present. In this material (Heinbecker, 12) the essential and sometimes only lesion present is a marked loss of cells in the paired paraventricular nuclei.

In the hypophysectomized dog the thyroid glands (figs. 4 and 5) are reduced in size. Microscopically the amount of colloid is greatly reduced and often markedly inspissated. The acini are small, many of them being without a lumen. Often large acidophilic cells are seen within and also apparently outside the acini. There is a considerable increase in fibrous tissue and in lymphoid infiltration.
The gland appears to lack the stimulus for the formation and the release of colloid.

The adrenal glands (figs. 4 and 5) are greatly diminished in size. Microscopically they show a marked increase in the amount of fibrous tissue in the capsule of the gland. There is a diminution in the width of the cortex. In dogs autopsied 2 years or more after hypophysectomy the fascicular and reticular layers may be almost completely atrophic, with the glomerular layer even somewhat taller than in the normal gland but the cells paler staining and more vesicular. After this longer period the medulla, too, becomes appreciably altered. It is atrophied, with the remaining cells showing marked degenerative changes. Earlier, i.e., 6 to 12 months after hypophysectomy, the cortex may simply be narrowed in all its layers while the medulla may show little obvious change.

In the female the ovaries (figs. 4 and 5), the breasts and the uterus show marked gross atrophy. Histologically there is seen a failure of the follicles to mature. In the dogs sacrificed after 2 years the interstitial tissue is atrophic, a condition not apparent in the dogs sacrificed early. In the male the testes are atrophied to one-third or less of their normal size. On microscopic examination
there is marked atrophy of the glandular elements with a failure of spermatogenesis. In the dogs sacrificed 2 years or more after operation the interstitial tissue also is obviously diminished in amount.

The pancreas (figs. 4 and 5) grossly show variable changes. On microscopic examination the acinar tissue is normal. The islets in some show definite hypertrophy and hyperplasia, in others there may be an atrophy with an apparent diminution in the number of the islets, in others no obvious deviation from the normal is apparent. It is the impression of the authors that the changes in the pancreas are probably the result of changes in other structures rather than a direct expression of the loss of the glandular hypophysis.

The liver in healthy operated dogs is grossly normal. Microscopically the liver cells are well preserved except when before sacrifice there has been some weight loss and a decrease in food intake when fatty infiltration may be marked. With increasing time after operation there is an increase in fibrous tissue throughout the liver.

Many of the above described effects of hypophysectomy have been noted also by others. They are described in order to compare the effect on similar structures of a hypothalamic puncture in the posterior hypothalamus so placed that degeneration of the paraventricular nuclei follows. The hypophysis is disturbed as little as possible but the stalk must be severed to approach the posterior hypothalamus by this route. In such a dog the size of the remaining glandular hypophysis may be almost normal or reduced by as much as 50 per cent, depending on the degree of trauma at operation. The hypophysis remaining is adequate to insure a normal insulin tolerance and a normal renal blood flow (White, Heinbecker and Rolf, 13).

Examination of the glandular hypophysis reveals in all instances an almost complete loss of the basophil cells, the few remaining ones being deprived of their granules (fig. 8). The eosinophils are relatively increased in number because of the loss of basophils. The possibility of an increase in eosinophils exists but no count to determine their actual number has been made. The chromophobes are not abnormal.

The gross and microscopic appearances of the thyroid, the pancreas and the liver are similar to those exhibited in these organs after simple or total hypophysectomy (figs. 4, 5 and 9). The ovaries show some atrophy and a suppression of the normal maturation of their follicles, the testes fail to show normal spermatogenesis but the interstitial tissue of the gonads appears normal. The greatest difference occurs in the adrenals. Here there is no gross or microscopic atrophy as occurs after hypophysectomy. Insufficient material is available to answer the question as to whether or not there is a hypertrophy. If there is, it is not sufficiently marked to be obvious.

The distribution of the fatty deposits is similar to that seen in extremely obese normal dogs or in obese dogs after hypophysectomy. The use of special stains has shown the abnormal deposits of fat in the skin to be composed of neutral fat and cholesterol (Liebermann-Burchardt reaction).

Functional studies. The blood glucose values (Somogyi method) after 16 to
24 hours of fasting for 15 normal dogs based on 2 to 8 observations for each dog varied from 80 to 65 mgm. per cent with an average value of 76 mgm. per cent. Values obtained for the fasting blood glucose of 8 simple hypophysectomy dogs with 2 to 8 observations on each dog varied from 79 to 50 mgm. per cent with an average value of 65 mgm. per cent. Similar observations on 6 total hypophysectomy dogs gave values from 67 to 55 mgm. per cent with an average of 60 mgm. per cent. For 7 puncture dogs the values ranged from 87 to 55 mgm. per cent with an average of 72 mgm. per cent. There is a greater fluctuation in the values for the puncture and simple hypophysectomy dogs than for normal dogs. Total hypophysectomy dogs have a somewhat lower blood glucose level than simple hypophysectomy dogs but the values also fluctuate markedly. Our observations confirm the fact established by others that loss of the glandular hypophysis results in a rapid fall in the blood glucose level with fasting. Puncture dogs on the other hand withstand fasting without the development of dangerously low hypoglycemic levels.

The insulin sensitivity of simple and total hypophysectomy dogs is greatly increased, that of the puncture dogs is unchanged.

The instability of the blood glucose levels in hypophysectomy and puncture dogs is regarded as a significant factor in causing such dogs to experience hunger. Hunger is manifested most clearly by the puncture dog.

Evidence of increased activity in the hypothalamus and other regions of the brain stem during the downward shift of the blood glucose level following the administration of insulin has been presented by Bartley and Heinbecker (14). According to Kabat et al. (15) stimulation of the hypothalamus results in inhibition of peristalsis of the stomach and of the small intestine. It would be reasonable to expect that on excitation of the brain stem of a dog in which the inhibitory region, i.e., the hypothalamus, had been functionally eliminated, there would occur increased peristalsis of the stomach and small intestine because activity of the exciting or parasympathetic system would be facilitated. The excitation of cells in the brain stem caused by a downward shift of blood glucose could also directly stimulate the cells through which the sensation of hunger is experienced. They, in turn, could be expected to activate the neural mechanism through which the peristalsis of the stomach and of the small intestine is increased as it is during hunger.

The cause of the rapid depression in blood glucose level in hypophysectomized dogs is to be found in their high tissue utilization of carbohydrate (Russell, 16), their insulin sensitivity and their depressed adrenal cortical function. The greater ability to utilize carbohydrate presumably would decrease the use of fat and permit its storage, particularly when the general metabolic demands are lessened because of a depression of thyroid and gonad function.

In puncture dogs the adrenal cortex does not atrophy. The ability of the blood sugar level to be maintained at a higher level on fasting than in hypophysectomized dogs is taken as evidence of functional capacity of the adrenal cortex. The evidence that puncture dogs are sensitized to exogenous adrenal cortical hormone in its influence on renal blood flow (Heinbecker, Rolf and White, 18)
cannot be regarded as final evidence that they are sensitized to it as a modifier of carbohydrate metabolism but it suggests the possibility. Further evidence in support of the concept of exaggerated or unbalanced function of the adrenal cortical hormone has been furnished by McQuarrie et al. (18) in their study of cases of Cushing's syndrome. In such cases, some associated with an adrenal tumor and some without such an association, the blood sodium level has been found elevated, the blood potassium level depressed. In similar cases not due to an adrenal tumor the primary pathology has been found to be an atrophy of the paired paraventricular nuclei in the hypothalamus (Heinbecker, loc. cit.). The effect of the adrenal cortical hormone, increased because of its increased amount in tumor cases and because of a hormonal imbalance in the others, could be expected to increase gluconeogenesis and directly or indirectly to increase fat formation from glucose and aid in its storage. Evidence has been presented by Brobeck et al. (19) to show that in rats which became obese after a hypothalamic injury, the conversion of carbohydrate to fat is increased.

There is no experimental evidence to indicate how extracts of the adrenal cortex lead to increased fat storage. It may be that fat storage is increased because the greater amount of carbohydrate available through gluconeogenesis lessens the necessity for fat utilization and thereby leads to its storage. In puncture dogs the caloric requirements too are lessened because of a depression of thyroid and gonad functions resultant from a loss of basophil cells in the hypophysis. The circumstances are such that food intake is well maintained and food requirements are lessened; under them obesity would be expected to follow.

Liver glycogen values 16 to 24 hours after the last food intake were obtained in a small number of dogs. In 1 normal dog the liver glycogen was 9.12 mgm. per cent; in 1 simple hypophysectomy dog, 7.1 mgm. per cent; in 1 total hypophysectomy dog, 6 mgm. per cent; in 4 puncture dogs, 9.3 mgm. per cent, 5.4 mgm. per cent, 6.8 mgm. per cent and 5.8 mgm. per cent, respectively, the last being a 3 months-old puppy. The saponifiable liver fatty acid for the normal fat dog was 0.297 mgm. per cent; for the simple hypophysectomy dog, 5.35; for the total hypophysectomy dog, 4.62 mgm. per cent; for 3 of the puncture dogs, 3.73 mgm. per cent 4.6 mgm. per cent and 1.67 mgm. per cent, the last reading being for the 3 months-old puppy. The liver glycogen values indicate a high carbohydrate reserve, adequate to permit a minimum utilization of fat for metabolic needs. The livers did not show fatty infiltration on microscopic examination.

Blood cholesterol values (modified Bloor method) for 13 normal dogs averaged 140 mgm. per cent; for 3 simple hypophysectomy dogs, 223 mgm. per cent; for 5 total hypophysectomy dogs, 299 mgm. per cent; and for 10 puncture dogs, 194 mgm. per cent. From these results it appears that obesity in adult dogs is associated with an elevated blood cholesterol but there is no correlation between the degree of obesity and the degree of elevation of the blood cholesterol. The

3 The tissues sensitized include the cells of the glandular hypothysis.
fatter puncture dog has a lower blood cholesterol than the fat total hypophysectomy dog.

It is of interest to note that hypophysial extract\(^4\) lowered blood cholesterol in simply hypophysectomized fat dogs from an average value of 250 mgm. per cent to 110 mgm. per cent. It did not change the blood cholesterol of puncture or of normal dogs appreciably. Adrenal cortical extract (Upjohn\(^5\)) given subcutaneously 4 to 8 cc. daily elevated the blood cholesterol of simply hypophysectomized fat dogs from an average value of 223 mgm. per cent to 315 mgm. per cent; of totally hypophysectomized fat dogs from an average value of 299 mgm. per cent to 460 mgm. per cent; of fat puncture dogs from an average value of 194 mgm. per cent to 315 mgm. per cent. This dosage did not alter the normal dog's blood cholesterol. Thyroid extract, 1/10 gram per kgm. of body weight per day for 14 days, lowered the blood cholesterol of fat puncture dogs from an average value of 194 mgm. per cent to 135 mgm. per cent; of normal dogs from an average value of 140 mgm. per cent to 80 mgm. per cent.

Observations were made to estimate the effect of the various operative procedures on food intake on 2 dogs in each class. The amount of food taken when given in unlimited amount for a week before operation was compared with that eaten at various periods after operation. The results indicate that under such circumstances both normal and operated animals eat large quantities of food but no striking difference was noted between the normal and the various operated classes after operation. Any 20 to 30 kgm. dog given unlimited food will eat between 1 to 2 kilos of meat plus 0.25 to 0.50 kilo of dog chow per day. During the period of rapid weight gain in operated dogs their appetite is particularly good, that of the puncture group always being striking. About 1 dog in 3 of the simple and total hypophysectomy groups and of the puncture groups after 1½ or more years of good health and weight gain will suffer a diminution in their water and food intake and become emaciated. At autopsy the precipitating cause invariably found has been a basilar meningitis particularly marked in the region of the hypothalamus. Such dogs show fatty degeneration of the liver. The islets of the pancreas frequently are markedly diminished in number and size. It is interesting to note that dogs previously exhibiting marked diabetes insipidus because of a proven loss of cells of the supraoptic nucleus nevertheless under such circumstances lose their thirst and have a normal or diminished urine output.

It is felt that our findings are consistent with the interpretation of Kabat and his associates (loc. cit.) that obesity in the rat develops on the basis of an increase in food intake but dogs do not exhibit the same degree of increase in hunger as do rats after appropriate hypothalamic lesions.

**Pathogenesis of experimental obesity.** Conditions which lead to a degree of obesity which surpasses that which occurs spontaneously in some normal dogs

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\(^4\) Preloban Niphanoid supplied through the generosity of the Winthrop Chemical Company.

\(^5\) The adrenal cortical extract was supplied in part through the kindness of the Upjohn Company.
with caging or age, and in simple or total hypophysectomy dogs, exist in the puncture dog. Two essential features, the gross normality of the adrenal glands and the increased sensitivity to exogenous adrenal cortical hormone, set this class of dog apart and are considered responsible for the abnormal storage of fat. Long et al. (20) have shown that adrenal cortical hormone is capable of augmenting gluconeogenesis. The additional carbohydrate formed thereby would be expected to lead to a greater utilization of carbohydrate for necessary bodily requirements and to lessen the demand for fat. Under such circumstances any excess of ingested fat or fat formed from excess carbohydrate intake or formation would be available for storage.

Support for the concept that adrenal cortical hormone leads to fat storage is found in the work of Hewer (21) who showed that the administration of lipoid extract of the adrenals of beef to rats results in obesity. Similar evidence has been presented by McKinley and Fisher (22) and by others.

Clinical evidence also is available to indicate that adrenal cortical hormone is concerned in the development of obesity. Thus in cases of adrenogenital syndrome due to an adrenal tumor, abnormal obesity is characteristically present.

Additional factors which doubtless aid in the speed of development of obesity not only in puncture dogs but also in the simple and total hypophysectomy dogs are the depression of thyroid and gonad activity.

It has been found by Biedl (23) and ourselves (unpublished data) that total thyroidectomy in the dog leads slowly to a loss of weight, possibly in part because of a depression of appetite. In our experience the moderate depression of thyroid activity diminishes bodily requirements for nutritive material but permits a maintained food intake and therefore storage of fat can and is known to occur. It has been established in the human that the weight increase in hypothyroidism is not only due to fat but also to retained water. The depression of gonad activity likewise is known to lead to a depression of the basal metabolic rate of about 20 per cent (Loewy and Richter, 24). With a maintained food intake storage of fat is known to occur.

Because of the great increase in fat storage which can occur spontaneously in the apparently normal dog it seems unnecessary to postulate an abnormal mechanism for the formation and storage of fat in our altered dogs. No evidence for any such alteration in metabolism has been found, all differences being quantitative rather than qualitative. Genetic factors undoubtedly exist but the manner in which they exert their influence is unknown. In man the exaggerated influence of or absence of certain hormones seems to some degree to determine the locale of fat storage. For example, in Cushing’s syndrome the fat storage occurs primarily in the face, neck and trunk whereas in hypogonadism it is chiefly about the pubis and the hips. No such differences have been observed in the various classes of dogs studied in this research.

Our results have indicated a more rapid development of obesity when the supraoptico-hypophysial system innervating the pitressin-forming tissue was totally or nearly totally inactivated; when total or nearly total diabetes insipidus exists. The appearance, activity and blood cholesterol values, especially of the
puncture dogs, do not indicate that the loss of pitressin lowers the metabolic rate. Possibly the explanation for the rapidity of their fattening is to be sought in evidence presented by Britton and Corey (25) and by others, which indicates that there is an antagonistic action between pitressin and the hormone from the adrenal cortex. It is conceivable therefore that the hormone of the adrenal cortex may become more effective when pitressin is lacking. Additional support for this concept is found in the clinical evidence that in Cushing's syndrome due to an adrenal cortical tumor, polyuria is frequent. In such a case the supra-optic and paraventricular nuclei have been shown to be histologically normal (Heinbecker, loc. cit.). Under such circumstances it is felt that the secretion from the adrenal tumor probably is responsible directly for the neutralization of the effect of pitressin in preventing polyuria. As stated above another possible explanation is to be found in our anatomical evidence that following removal of the median eminence there is a loss of cells, particularly in the rostral division of the paraventricular nuclei. Possibly some of the cells lost are of the class which normally send their fibers caudally to exert an influence similar to that exerted by other paraventricular cells in the caudal division of the nucleus, the loss of which ultimately causes the development of obesity. If, however, they are concerned only in activating pituicytes in the median eminence their influence on obesity would have to be considered indirect through an unbalanced increase in the effect of the adrenal cortical hormone.

It is deemed unlikely that the fibers going to the median eminence from the paraventricular nucleus cause the secretion of a hormone whose loss is responsible for the disappearance of basophil cells from the hypophysis of puncture dogs, because of the knowledge that marked diabetes insipidus may exist in humans without obesity and without a suppression of ovarian function such as is associated with hyalinization of the basophil cells. Their presumed intactness in such cases would argue against the loss of pitressin being responsible for the hyalinization of the basophil cells. This is also borne out by the fact that stalk section, which results in considerable loss of pitressin, does not in itself lead to hyalinization of the basophil cells of the hypophysis.

It is of interest to note that the cells of the two nuclei, supraoptic and paraventricular, are of similar cytological character. Destruction of one of them, the supraoptic, leads to thirst and to polydipsia, destruction of the other, the paraventricular, to hunger and to polyphagia. The rostral division of the paraventricular may play a dual function. Some of the cells innervate, in part, the median eminence and therefore probably are concerned in the secretion of pitressin and thereby in the regulation of water balance while others as stated above may have an influence on obesity. The frequent accompaniment of pathological obesity with mild disturbances in water balance may find an explanation in such a dual function of the paraventricular nucleus.

There remains unanswered the question as to how the loss of the paraventricular nucleus leads to regressive changes in the gonade and to changes in carbohydrate metabolism and fat storage. These effects probably are initiated by changes in the effectiveness of the adrenal cortical hormone directly on carbo-
hydrate metabolism and indirectly through an influence on the basophil cells of the hypophysis. It is fairly certain that it is not through nervous pathways connected with the sympathetic or parasympathetic nervous system, because no such changes are effected by complete sympathectomy or by section of the vagus nerves beneath the diaphragm. It seems more probable that the fibers which pass caudally from the paraventricular nucleus innervate cells within the brain stem which secrete a hormone which directly or indirectly influences the adrenal cortex or the basophil cells of the hypophysis. It is suspected that this may be in the region of the epiphysis because clinical and experimental evidence, admittedly not conclusive, has pointed to this region of the nervous system as having an influence on fat storage and on the sex organs. Investigations are in progress to trace the destination of the fibers descending from the paraventricular nucleus.

SUMMARY

Experimental evidence is presented to show that obesity in the dog results from bilateral destruction or retrograde degeneration of the paired paraventricular nuclei, particularly of their caudal portions.

Marked obesity results when destruction or denervation of the neurohypophysis and destruction or retrograde degeneration of the paired caudal paraventricular nuclei co-exist.

Removal of the pars distalis itself in the adult dog results in a less marked and less rapid development of obesity but there does occur a slow weight increase which becomes marked with time.

The presence of the pars distalis in animals with the supraoptic and paraventricular nuclei destroyed or degenerated is favorable to the rapid development of marked obesity.

Destruction or denervation of the caudal portion of the paraventricular nuclei leads to changes in the body which increase the food intake. The organism and in particular the cells of the glandular hypophysis are rendered sensitive to the adrenal cortical hormone and probably because of this there results a marked loss of basophil cells in the glandular division of the hypophysis.

This results directly in changes in the thyroid and in the gonads and probably indirectly in the islet cells of the pancreas. The adrenals do not atrophy. The alterations in the metabolism effected by such changes result in the accumulation of fat in various tissues of the body. Total and simple hypophysectomy dogs show in addition to changes in the thyroid, the gonads and the pancreas an atrophy of the adrenal cortex. This may explain the fact that while they become obese the rate of accumulation of their fat and its amount are less than in the "puncture dogs."

Obesity is considered to result because of an excess of food intake over food requirement. The food intake may be excessive because of a reduction in metabolic needs effected through a depression of thyroid or of gonad activity or of both together, while hunger is maintained. It may be excessive in answer to an exaggerated hunger stimulated by a downward shifting blood glucose level
caused by the exaggerated consumption of carbohydrate in the tissues as in
hypofunction of the hypophysis; or by effects resulting from exaggerated influ-
ences from the adrenal cortical hormone following an appropriate hypothalamic
lesion. The changes in metabolism leading to obesity are quantitative rather
than qualitative.

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