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Review

The adipokinetic action of polypeptide and amine hormones upon the adipose tissue of various animal species

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I. INTRODUCTION

"Adipokinetic" signifies the capacity of a substance to cause the release of triglyceride stored in adipose tissue, with subsequent transport of the mobilized lipid by the blood to other organs of the body. The concept that certain hormones possess adipokinetic activity[1] was introduced by the investigations of Best, Barrett, and co-workers (1, 2), and of Stetten and Salcedo (3) during the period 1936–1944. These investigators described a redistribution of triglyceride from adipose tissue to the liver in rats and in mice injected with pituitary extract. Further research on this subject has proceeded in three phases:

(*a*) From 1949 to 1957, studies were made on the adipokinetic activity of various pituitary fractions, as measured by the capacity of each to cause an increase in the hepatic lipids of susceptible species[2] (4–9). The production of fatty liver in certain species by epinephrine was also demonstrated (review [10]).

(*b*) In 1956, Gordon and Cherkes (11) and Dole (12) presented evidence that triglyceride is mobilized from adipose tissue in the form of unesterified (free) fatty acids (FFA), which circulate as an albumin-FFA complex. It was then found that the catechol amines (11, 12) and certain pituitary hormones (13–15) cause an acute rise in circulating FFA level in intact animals of susceptible species.

(*c*) Recent studies (1958–1962) have demonstrated that the catechol amines (16, 17), various pituitary polypeptides (14, 18–22), and the pancreatic polypeptide glucagon (23, 24) stimulate the production of FFA *in vitro* by the isolated adipose tissue of susceptible species. The *in vitro* technique has facilitated the identification (14, 18, 19, 21–25) and purification (21) of adipokinetic hormones and has made possible observations on the metabolic changes within adipose tissue following stimulation by these hormones (reviews [26–29]).

These *in vitro* experiments have revealed that the effect of adipokinetic hormones upon the fat cell is complex, involving alterations in the rates of such processes as triglyceride synthesis, triglyceride hydrolysis, glucose uptake, and glucose oxidation; and increases in the activities of phosphorylase and of one or more of the intracellular lipases (26–29). The hormonal effect responsible for the mobilization of FFA is believed to be the increase in the activity of an intracellular lipase, with a resulting increase in the rate of hydrolysis of the triglyceride stored in the fat cell and a rise in the intracellular concentration of FFA (15, 30–34).

In vivo experiments (35-38) have demonstrated that this sequence is followed by the discharge of FFA from adipose tissue into the blood, with a resulting rise in the circulating FFA level. In the intact animal, the increase in serum FFA concentration occurs within 30 min after subcutaneous injection of adipokinetic peptides and persists for 2–12 hr. Considerable amounts of the FFA mobilized in this way are taken up by the liver and kidney, where they are reesterified and temporarily stored as triglyceride. The concentration of triglyceride in the liver and in the kidney

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[1] The term "lipolytic activity" is also in use to denote this property.

[2] The differences in the responsiveness of various species to the adipokinetic hormones will be considered in detail in a subsequent section of this review.

increases progressively for several hours after the injection. Depending on the magnitude and duration of the mobilization of FFA and of the accumulation of triglyceride in the liver, there may ensue 8–12 hr after the injection, a discharge from the liver[3] into the blood of low-density lipoproteins, rich in triglycerides, with a resulting hyperlipemia. A similar sequence of events appears to be produced by catechol amines administered by prolonged intravenous infusion (38) or by subcutaneous injection of a repository preparation (39).

Two puzzling features have emerged from recent studies on adipokinetic hormones: (*a*) at least 10 different substances possessing adipokinetic activity are found in the anterior or posterior pituitary gland, in the sympathetic nervous system, or in the pancreas (ACTH,[4] TSH, α-MSH, β-MSH, arginine vasopressin, "fraction H" ["peptide II"], "peptide I," epinephrine, norepinephrine, and glucagon [14, 16–19, 21–25, 40, 41]; and (*b*) the adipokinetic potency of each of these hormones varies markedly in different mammalian species [14, 20, 21, 25, 28]). This review will present the information now available on (*a*) methods for measuring the adipokinetic activity of hormones; (*b*) the multiplicity of hormones with adipokinetic activity; (*c*) the variation in their adipokinetic potencies in eight mammalian species, namely rabbit, guinea pig, hamster, rat, mouse, pig, dog, and man.

II. METHODS OF MEASURING THE ADIPOKINETIC ACTIVITY OF HORMONES

1. In vivo *Assay Methods.* The sequential alterations in lipids of adipose tissue, blood, liver, and kidney following injection of an adipokinetic hormone in an animal of a susceptible species provide several possible indicators of the animal's response to the adipokinetic action of the hormone. Three commonly employed indicators of response are the increase in circulating FFA concentration within the first hour after injection of hormone (13, 14, 21, 42), increase in hepatic esterified lipids 3–6 hr after injection (4–8), and increase in circulating esterified lipids 12–24 hr after injection (21, 37, 41).

Control of endogenous factors that influence FFA mobilization is difficult in assays conducted in intact animals. The following example can be cited. Fasting leads to a progressive rise in circulating FFA level, first detectable about 4 hr after the last meal (11, 12, 43–45). Injection of growth hormone at the beginning of the fast accentuates this rise (44–46). This growth hormone-induced increment in FFA level is promptly abolished by the intravenous injection of glucose. Other pituitary substances to be discussed below (e.g., ACTH, α- and β-MSH) produce a more rapid increase in circulating FFA concentration (detectable within 30 min after injection), which is not dependent on the fasting state and which is not suppressed by injection of glucose (13, 14, 21). Unlike such adipokinetic substances as ACTH, α- and β-MSH, growth hormone has little or no effect upon the production of FFA by slices of adipose tissue *in vitro* (18, 25, 47, 48). Accordingly, it seems probable that the influence of growth hormone on circulating FFA concentration in the fasting animal results indirectly from metabolic alterations produced by this hormone in other organs, rather than from a direct effect of the hormone upon adipose tissue. The theoretic possibilities should also be mentioned that a substance might cause mobilization of FFA in an intact animal by stimulating secretion of endogenous adipokinetic hormones from the pituitary gland or sympathetic nervous system, or by enhancing the responsiveness of the adipose tissue to these hormones. Such mechanisms may be involved in the production of lipemia in the rabbit by cortisone (49), and in birds by estrogens (50). Pretreatment of normal animals with L-triiodothyronine enhances the responsiveness of the isolated adipose tissue to epinephrine (51).

Although *in vivo* assays are less precise and more laborious than *in vitro* assays, they may provide information not afforded by the latter technique. The effects of an adipokinetic hormone *in vivo* are determined not only by the adipokinetic potency of the hormone upon the adipose tissue (which can be measured with precision by the *in vitro* technique), but also by the distribution of the hormone in the body and by the rate of degradation and excretion of the hormone by organs other than adipose tissue. Furthermore, experiments in intact animals permit study of the metabolic and anatomic changes that occur in other organs following mobilization of FFA from adipose tissue (36–38, 40, 52, 53). It is desirable, therefore, that the adipokinetic properties of the hormone be examined *in vivo* as well as *in vitro*.

2. In vitro *Assay Methods.* When slices of epididymal, perirenal, or mesenteric adipose tissue are incubated in Krebs-Ringer medium, FFA are produced within the tissue at an accelerated rate in response to addition of adipokinetic hormone to the medium (16–18). If plasma albumin is not present in the medium, the newly produced FFA accumulate only within the

[3] R. L. Hirsch, unpublished observations.

[4] The following abbreviations are employed: ACTH, adrenocorticotropin; TSH, thyroid-stimulating hormone; α-MSH and β-MSH, α- and β-melanocyte-stimulating hormones.

tissue slice and do not appear in the medium (17–19, 25, 54). If albumin is present, the major proportion of newly formed FFA leaves the cells and accumulates in the medium in the form of the albumin-FFA complex.[5] The course of the response to adipokinetic peptides and amines is different in the albumin-free and in the albumin-containing systems (25). In the former system, the concentration of FFA in the tissue increases rapidly for 60–90 min and then achieves a plateau concentration. In the latter system, the total concentration of FFA in the tissue and in the medium continues to increase throughout a 3-hr incubation period.

Most comparisons of the adipokinetic potencies of different hormones *in vitro* have been conducted with the albumin-containing system and have been designed to determine the minimal effective dose of each hormone under study (14, 15, 17–19, 21, 22). The accumulation of FFA in the medium, in the tissue, or in the medium plus tissue, has represented the response of the adipose tissue to the hormone. In addition to estimation of the minimal effective dose, a delineation of the entire dose-response curve for the action of each adipokinetic hormone upon a particular adipose tissue is desirable. The suitability of different techniques for defining dose-response curves for adipokinetic substances has recently been investigated in experiments with adipose tissue from the rabbit and guinea pig (25). In the albumin-free system, the "plateau" concentration of FFA in the adipose tissue (see above), after 3 hr of incubation, exhibited a reproducible, sigmoid relationship to the logarithm of the concentration of adipokinetic hormone present in the medium at the beginning of the incubation period. In the albumin-containing system, the concentration of FFA in the medium, in the tissue, or in the medium plus tissue, at the end of a 3-hr incubation, was likewise found to be dependent upon the concentration of adipokinetic hormone in the medium, but a reproducible relationship between response and dose could not be established. Satisfactory methods for the determination of dose-response curves of adipokinetic hormones are desirable because of the practical and theoretic applications of the dose-response relationship (59–61).

Estimations of the minimal effective doses of adipokinetic hormones upon the adipose tissues of various species have been made in several different studies, and considerable variation in the results is apparent. Thus, values reported for the minimal effective dose of ACTH upon rat adipose tissue vary from 1.0 μg/ml to 0.0001 μg/ml[6] (15, 18, 25, 47). These variations doubtless result from differences in experimental technique that increase or decrease the sensitivity of the assay; e.g., electrolyte composition and albumin content of the medium (54), presence in the albumin preparation of FFA and of other contaminants (28, 58), age (62) and nutritional status (16, 63) of the animal, type of adipose tissue used (epididymal, perirenal, mesenteric, or subcutaneous) (63), and purity of the hormone preparation under study. Seasonal variations in the sensitivity of rat adipose tissue to ACTH have also been observed (64).

III. THE ADIPOKINETIC POTENCIES OF PITUITARY PEPTIDES, GLUCAGON, AND THE CATECHOL AMINES IN DIFFERENT MAMMALIAN SPECIES

1. *ACTH, TSH, α- and β-MSH, and Arginine Vasopressin.* Each of these recognized pituitary hormones exhibits adipokinetic activity *in vitro* at concentrations as low as 0.001–0.3 μg/ml upon the adipose tissue of certain mammalian species, but in other species exhibits no activity at the highest concentrations tested (10–100 μg/ml). A summary of the available information on species variation in the adipokinetic activity of these hormones, gathered from *in vitro* and *in vivo* experiments, follows:

Porcine ACTH: Activity in the rabbit, guinea pig, hamster, rat, and mouse; little or no activity in the dog, pig, or man (7, 13, 15, 18, 21, 25, 40, 65).

Bovine TSH: Activity in the guinea pig, rat, mouse, and dog; little or no activity in the rabbit, hamster, or pig (4, 19, 25).

Bovine α- and β-MSH: Activity in the rabbit, guinea pig, and dog; little or no activity in the hamster, rat, or pig (14, 22, 25).

Arginine vasopressin: Activity in the rabbit and guinea pig; little or no activity in the hamster, rat, pig, or dog (18, 25).

[5] Plasma albumin has the capacity to bind with varying degrees of affinity up to 27 moles FFA/mole albumin (review [55]). The plasma lipoproteins also have a capacity to bind FFA (55, 56), which has not been as precisely characterized but is many times less than that of plasma albumin. The possible effect of proteins other than plasma albumin in the *in vitro* system for assaying adipokinetic activity has not yet been reported.

Commercial preparations of bovine plasma albumin contain as much as 30 μEq FFA/g albumin. The sensitivity and reproducibility of the "*in vitro*" assay system containing albumin are greatly improved if the albumin has been freed from FFA by a prior extraction with isopropyl alcohol–heptane–N H_2SO_4 40:10:1 or with glacial acetic acid–isooctane (57).

Blackard and coworkers have demonstrated (58) the presence in bovine albumin preparations of contaminants of unknown structure that promote the oxidation of newly formed FFA to peroxides.

[6] In all of these studies, oxycellulose-purified ACTH (70–140 units/mg) was employed.

2. *"Fraction H"* (*"Peptide II"*). Pituitary "fraction H" was prepared from pig pituitaries by alkaline aqueous extraction, acetone precipitation, and ion exchange chromatography, the fractionation being guided by the capacity of the active material to cause lipemia in the rabbit (41, 66). Fraction H contains less than 0.8% of any of the recognized pituitary hormones (40, 41, 66). The active component in this fraction has the properties of a polypeptide (66), and is identical to "peptide II" subsequently isolated by a different technique from pig pituitaries by Astwood and coworkers (21, 67, 68) (see below). Porcine fraction H possesses high adipokinetic potency *in vitro* only upon rabbit adipose tissue among eight species so far examined (25, 40). It is weakly active upon guinea pig and chicken adipose tissue, and not detectably active upon the adipose tissue of the hamster, rat, mouse, pig, and dog. The pattern of species variation is the same for ovine fraction H, except that this preparation is weakly active upon rat adipose tissue (40).

3. *"Peptide I"*. Astwood and coworkers have identified, in a fraction of the acetic acid extract of pig pituitaries, two polypeptides (labeled by them "peptide I" and "peptide II"), which possess high adipokinetic potency upon rabbit adipose tissue (21, 67). These two substances differ slightly in electrophoretic mobility (21). Differences in molecular weight, in behavior on Dowex columns, and in immunochemical reactions have been reported (67, 68). Peptide II has been found to be identical with a major component in fraction H (21, 68). Systematic comparison of the physical, chemical, and biologic properties of peptides I and II are awaited in order to elucidate the relationship between these two pituitary preparations. Comparison of the pattern of species variation of peptide I with that of peptide II will be especially valuable in this connection. So far it has been observed (21) that porcine peptide I has high activity in the rabbit, but little or no activity in the rat, dog, or man.[7]

[7] Friesen, Barrett, and Astwood (21) found that the subcutaneous injection of a preparation of porcine peptides I and II in human subjects had little effect upon the serum FFA concentration. These investigators then prepared from human pituitary glands a fraction containing two components with the same electrophoretic mobility as porcine peptides I and II. The injection of this preparation also had little or no effect upon the serum FFA level in human subjects. Studies in the author's laboratory (40) have demonstrated that the adipokinetic components in porcine, ovine, and bovine fraction H differ one from the other in their "leak point" through IRC-50 resin. These differences suggest that this pituitary polypeptide may vary in structure, and consequently in isoelectric point and in electrophoretic mobility, in the pituitary glands of different species. Accordingly it is possible that the components isolated from human pituitary glands by Friesen and coworkers may not be the counterpart of peptides I and II as isolated from pig pituitary glands.

4. *"Lipid-Mobilizing Factor" of Seifter*. Seifter and Baeder reported (69) that a dialyzable material obtained from the posterior lobes of pig pituitary glands caused lipemia 1 hr after intravenous injection into fasting mice, guinea pigs, rabbits, or dogs. This material was reported to inhibit *in vitro* the lipoprotein lipase activity of postheparin plasma. The effects of this material upon FFA production by adipose tissue slices *in vitro* or upon the circulating FFA level *in vivo* have not yet been described. The biologic properties of Seifter's "lipid-mobilizing factor" differ from those of any of the pituitary adipokinetic components listed above with regard to the early onset of lipemia, the dependence of lipemia upon the fasted state, and the uniformly high activity described by Seifter and Baeder for this preparation in the mouse, guinea pig, rabbit, and dog.

5. *Adipokinetic Activity of Urine from Fasted Humans*. Chalmers and coworkers (70–72), extending the earlier observation of Weil and Stetten (73), have reported adipokinetic activity in urine obtained from fasting man, horse, dog, goat, and sheep, as indicated by the effect of subcutaneous injection of urine fractions upon the liver fat content of the mouse. Partial purification of the active component(s) in the urine of fasting humans has been described (70, 71). The resulting preparation is highly active *in vitro* upon rat adipose tissue. The chemical structure of the active component(s) is not known. Nevertheless, the reported absence of adipokinetic activity in the urine of fasting hypopituitary man (70, 71) suggests that the active material in the urine of fasting normal subjects may be derived from a pituitary polypeptide.

6. *Glucagon*. Glucagon possesses adipokinetic activity upon rat adipose tissue (23, 24). Its possible activity in other species has not yet been systematically studied.

7. *Epinephrine and Norepinephrine*. These amines exhibit considerable adipokinetic activity in the hamster, rat, mouse, dog, monkey, and man; but little or no activity in the rabbit, guinea pig, or pig (11, 12, 16, 17, 25, 42, 44, 45, 74).

A comparison of the *in vitro* adipokinetic potencies of ACTH, TSH, α-MSH, β-MSH, vasopressin, fraction H, epinephrine, and norepinephrine upon the adipose tissue of the rabbit, guinea pig, hamster, rat, pig, and dog is provided in Table 1, which is taken from reference (25). The following patterns of responsiveness may be noted:

(*a*) High degree of responsiveness to nearly all the adipokinetic pituitary polypeptides, and lack of responsiveness to catechol amines (rabbit and guinea pig).

TABLE 1. MINIMAL EFFECTIVE DOSE* OF 8 ADIPOKINETIC HORMONES UPON THE ADIPOSE TISSUE OF 6 SPECIES OF VERTEBRATES.

	Hormone							
Species	ACTH	α-MSH	β-MSH	Vaso-pressin	TSH	Fraction H	Epinephrine	Nor-epinephrine
Rabbit	0.1 μg/ml	0.1 μg/ml	0.01 μg/ml	0.1 μg/ml	NR	0.3 μg/ml	NR	NR
Guinea pig	3.0 μg/ml	10.0 μg/ml	10.0 μg/ml	3.0 μg/ml	1.0 μg/ml	100 μg/ml	NR	NR
Hamster	10.0 μg/ml	NR	NR	NR	NR	NR	0.1 μg/ml	0.3 μg/ml
Rat	0.1 μg/ml	NR	NR	NR	0.1 μg/ml	NR	0.1 μg/ml	0.01 μg/ml
Pig	NR†	NR	NR	NR	NR	NR	NR	NR
Dog	NR	<10.0 μg/ml‡	<10.0 μg/ml‡	NR	<10.0 μg/ml‡	NR	<0.1 μg/ml‡	<0.1 μg/ml‡

* The minimal effective dose is the smallest concentration of hormone that produces a statistically significant ($p < 0.05$) increase in FFA production. Experiments with rabbit, guinea pig, and hamster adipose tissue were performed with the albumin-free assay system; those with rat, pig, and dog adipose tissue employed the albumin-containing assay system.

† NR indicates no response to the highest concentration tested. The highest concentrations tested were 100 μg/ml for rabbit, guinea pig, and hamster; 10 μg/ml for rat, pig, and dog.

‡ A statistically significant effect was produced at this concentration, which was the smallest concentration of this hormone tested.

(*b*) Responsiveness to certain of the pituitary polypeptides, and also to the catechol amines (hamster, rat, and dog).

(*c*) Lack of responsiveness to either pituitary polypeptides or to catechol amines (pig).

IV. UNSOLVED PROBLEMS

The observations reviewed in the preceding sections reveal two unusual characteristics of the fat cell: (*a*) it responds in the same manner to a variety of hormones of diverse chemical structure (28), (*b*) the fat cells of the various mammalian species differ markedly in their responsiveness to each of these hormones. The following are some of the questions that are raised by these characteristics of the fat cell and that await clarification from future investigations.[8]

1. *Possible Similarity in the Chemical Structures of the Various Adipokinetic Hormones and in Their Mechanisms of Action.* A relationship in structure and in biologic properties between ACTH, α-MSH, and β-MSH is now recognized (77–79). Until recently, the ACTH-MSH group of peptides, TSH, arginine vasopressin, and the catechol amines have been regarded as unrelated in chemical and biologic properties. It is now evident that the fat cell responds with an increase in the rate of production of FFA to all of these hormones. In a recent study, no difference was found in the slopes of the dose-response equations representing the adipokinetic actions of ACTH, α-MSH, β-MSH, TSH, vasopressin, and fraction H upon rabbit and guinea pig adipose tissue (25). The pattern of metabolic alterations in rat adipose tissue exposed to ACTH or to epinephrine is identical in all respects so far examined (24–29). The adrenergic blocking agent phentolamine suppresses the responsiveness of rat adipose tissue not only to epinephrine, but also to ACTH (80). These observations suggest that the various adipokinetic hormones may possess a structural feature in common, which causes the same effect upon the fat cell. Correlation of structures and adipokinetic activities of various pituitary peptides has suggested that the arginine moiety is essential for adipokinetic activity (25).[9] Examination of the adipokinetic activities of series of synthetic peptides (14, 22, 77, 81) will eventually clarify the structure-activity relationship. The problem is complicated by the fact that the total structural requirement for adipokinetic activity varies in different mammalian species (see section 2 below).

A corollary implication is that the adipokinetic hormones, which appear to act by the same common mechanism to stimulate the production of FFA by the fat cell, may also act by the same or a closely related mechanism to produce their characteristic effects upon their respective "specific" target organs (e.g., adrenal cortex, thyroid, melanocyte, renal tubule) (28, 47, 75, 76). Evidence favoring the possibility of a common mechanism for the action of the various adipokinetic hormones upon the fat cell and upon their other target organs has come from studies on the mechanisms of actions of epinephrine, glucagon, ACTH, and vasopressin. Sutherland and coworkers have demonstrated (reviews [82, 83]) that epinephrine and glucagon cause the formation of adenosine 3′,5′-monophosphate (3′,5′-AMP) within the liver cell.

[8] The reader is referred to the reviews of Dr. F. L. Engel (28, 47, 75, 76) for a discussion of many of the topics considered in the following sections.

[9] It is conceivable that the basic amine group of the catechol amines, and the basic guanidine group of the arginine-containing adipokinetic polypeptides, might play analogous roles in the action of each of these two classes of hormone upon the fat cell.

This nucleotide has the property of activating liver phosphorylase, with resulting increase in the rate of glycogenolysis. Exposure of liver slices or homogenates either to epinephrine, to glucagon, or to 3′,5′-AMP augments the phosphorylase activity of the tissue preparation. Haynes then showed by *in vitro* experiments (84, 85) that ACTH causes an accumulation of 3′,5′-AMP and an increase in the activity of phosphorylase in the adrenal cortex. 3′,5′-AMP, like ACTH, augments the phosphorylase activity and rate of production of corticosteroids within the adrenal gland (85–87). Orloff and Handler then reported (88) that 3′,5′-AMP produces an effect similar to that of vasopressin upon the movement of water and sodium across the isolated toad bladder. Vaughan has reported (23) that epinephrine, glucagon, and ACTH cause an increase in the activity of phosphorylase in slices of rat adipose tissue. The observations up to this point are consistent with a role of 3′,5′-AMP in the mechanism of action of epinephrine and of glucagon upon the liver; of ACTH upon the adrenal cortex; of vasopressin upon the bladder; and of epinephrine, glucagon, and ACTH upon adipose tissue (83). Nevertheless, in experiments so far performed, 3′,5′-AMP has failed to stimulate the production of FFA by slices of adipose tissue from the rat (89), hamster, or rabbit.[10]

A different explanation for the sharing of adipokinetic activity by catechol amines and polypeptides has been suggested by Paoletti et al. These investigators have demonstrated the presence of norepinephrine in the adipose tissue of the rat and rabbit and have reported that the prior administration of reserpine, which depletes the adipose tissue of norepinephrine, suppresses the responsiveness of the rat's adipose tissue to ACTH (90, 91). Accordingly, Paoletti has suggested that norepinephrine plays a role in the action of ACTH in mobilizing FFA from adipose tissue. Edmonson and Goodman (92), however, were unable to reproduce the effect of reserpine described above. Furthermore, the observation that ACTH is highly active upon the adipose tissue of the rabbit and guinea pig, while norepinephrine and epinephrine are not detectably active in these two species (25), is not readily reconciled with the above hypothesis.

2. *Differences Between the Fat Cells of Various Species.* The adipose tissue of each of the six vertebrate species so far studied comprehensively shows a different pattern of responsiveness to adipokinetic hormones (25). Stated differently, the structural requirements for adipokinetic activity vary in each species. These differences in responsiveness to hormones may have resulted from changes in the composition of the fat cell that occurred during the evolution of the mammalian species. The following approaches to this subject may provide useful information:

(*a*) A possible phylogenetic pattern should be sought in the variations among the species in responsiveness to adipokinetic hormones. At present insufficient data are available to establish or disprove such a pattern.

(*b*) A possible correlation should be sought between the structural requirements for adipokinetic activity in each species and the structure of the homologous adipokinetic polypeptide hormones. It is possible that evolutionary changes may have occurred in the structure-activity requirement of the fat cell that were related to evolutionary changes in the structure of the adipokinetic hormones. For example, it has been found that rabbit adipose tissue, unlike that of the guinea pig or rat, is unresponsive to bovine TSH (25). It is nevertheless possible that rabbit adipose tissue may respond to rabbit TSH. In support of this possibility are the demonstration of species differences in the structure of several of the pituitary hormones (93), and the evidence favoring an analogous explanation for species variation in the responsiveness to growth hormone preparations (44, 45, 93, 94). To test this hypothesis, observations on the effect of each adipokinetic hormone upon the homologous adipose tissue will be required. In each of the three such experiments already performed (viz porcine ACTH and porcine fraction H upon pig adipose tissue (25, 40), and human ACTH upon human adipose tissue [65]), a lack of effect of the homologous preparation has been found. These negative findings, together with the fact that the above concept is not applicable to species variation in responsiveness to the catechol amines, suggest that this concept will not provide a general explanation for species differences in the fat cell's responsiveness to polypeptide hormones. Inasmuch as the protein hormones of the pituitary gland appear to have undergone more extensive alterations in chemical structure during evolution than the smaller polypeptide hormones of this gland (92), the possible applicability of this hypothesis to the adipokinetic properties of TSH deserves special consideration.

(*c*) The cellular mechanism(s) responsible for species differences in the responsiveness of the fat cell to hormones should be investigated. Some possible determinants of the degree of responsiveness are: the capacity of the fat cell to take up the hormone from the extracellular fluid (95), the presence or absence of the appropriate cellular "receptor" (96), and the action of intracellular enzymes that may alter the structure

[10] Unpublished observations in the author's laboratory.

of the hormone with resulting gain or loss of adipokinetic activity (97).

3. *Possible Physiologic Functions of the Adipokinetic Activity of Polypeptide and Amine Hormones.* The studies reviewed in Parts I and III have demonstrated that the injection of exogenous polypeptide or amine hormones with adipokinetic activity in intact animals of susceptible species causes the mobilization of FFA from the adipose tissue into the circulation. Little is known, however, concerning the possible physiologic role of the endogenously secreted hormones in the regulation of FFA mobilization. It must be established in each species which of these hormones may be secreted from the pituitary gland or sympathetic nervous system in an amount sufficient to stimulate the adipose tissue, and under what conditions and following what stimuli this possible endocrine function may be called into play. Facts and speculations relevant to these topics are discussed below.

Comparison of the data obtained *in vitro* on the adrenocorticotropic action of ACTH upon slices of rat adrenal glands (98), and on the adipokinetic action of this hormone on slices of rat adipose tissue (15, 47, 99), show that the minimal effective concentration of ACTH required to produce a response is of the same order of magnitude for each of these two tissues. This conclusion supports the notion that the amount of ACTH secreted physiologically in the intact rat, which is sufficient for the maintenance of normal adrenal cortical function, may also be sufficient to influence the rate of mobilization of FFA from the adipose tissue in this species (99).

One approach to this subject has been to study the effect of pharmacologic blockade of the sympathetic nervous system, or of hypophysectomy, upon the capacity of an animal to mobilize FFA. So far the effect of these measures has been studied only upon the mobilization of FFA induced by starvation.

(*a*) Havel and Goldfien observed (100) that the administration to the fasting dog of hexamethonium or dibenamine caused an immediate reduction in the circulating FFA level. They interpreted this as evidence that the endogenously secreted catechol amines play a role in the mobilization of FFA in the fasting intact dog. In the rat, however, Goodman and Knobil found (101) that hexamethonium, ergotamine, or dibenzyline did not modify the serum FFA response to fasting. Nevertheless, Wertheimer and colleagues found (63) that the increase in the rate of production of FFA by slices of adipose tissue from fasting rats, as compared to those from fed rats, is reduced if dibenzyline is administered to the fasting animal.

(*b*) The hypophysectomized monkey (102) or rat (43) exhibits during fasting an increase in serum FFA concentration that is 50–70% as great as that of the intact animal. Slices of adipose tissue from the fasting hypophysectomized rat release FFA at only 15% the rate of slices from the fasting intact rat (103). These observations indicate that the mobilization of FFA during fasting in the monkey and rat is initiated by factors other than the adipokinetic pituitary hormones (43), but suggest that these hormones might act to enhance the response of the fat cell to the fasting state. The observation of Chalmers and coworkers that fasting causes the appearance of adipokinetic activity in the urine of normal man, but not in the urine of the hypopituitary subject (70, 71), supports this possibility. However, other interpretations of the reduced capacity of the hypophysectomized monkey or rat to mobilize FFA during fasting are possible (43). The capacity of growth hormone to intensify the effect of fasting upon the circulating FFA level has already been mentioned (44–46, 102). In addition, it has been established that, in the absence of adrenal cortical steroids, the responsiveness of the adipose tissue of the mouse or rat to ACTH (4, 6–8, 99), and of the dog to epinephrine (104), is diminished. Furthermore, removal of the adrenals in the rat is followed by a 30–50% reduction in the effect of fasting upon the serum FFA level, which is corrected by the administration of hydrocortisone (43). These observations, taken together, indicate that the capacity of the fat cell to release FFA in response to fasting or to adipokinetic hormones is curtailed in the absence of adrenal cortical steroids. The mechanism of this steroid effect upon the fat cell is not known. The reduced production of adrenal cortical steroids in the hypophysectomized animal, rather than the ablation of pituitary adipokinetic polypeptides or growth hormone, may be responsible for the diminished capacity of the hypophysectomized monkey or rat to mobilize FFA during fasting (43). It is, of course, possible that the mechanisms involved in the mobilization of FFA in response to fasting vary in different species. It would be of interest to examine the effect of sympatholytic drugs and of hypophysectomy upon the mobilization of FFA during fasting by such species as the rabbit or guinea pig, in which the pituitary polypeptides rather than the catechol amines appear to be the principal adipokinetic agents (25).

Present concepts of the intermediary metabolism of the fat cell and the available experimental data (26–29) suggest that in all mammalian species a decrease in the availability of insulin or glucose to the fat cell will be found to cause an increase in the rate of discharge of

FFA.[11] Goodman and Knobil have suggested (43) that a reduction in the rate of secretion of insulin is the factor initiating the mobilization of FFA that occurs in fasting in all species so far examined. In addition to the influence of insulin, alternate endocrine mechanisms (i.e., the adipokinetic pituitary polypeptides and the catechol amines) appear to be present in most mammalian species (25) to make possible the acute mobilization of FFA in the nonfasting state without any general alteration in carbohydrate metabolism.

A possible explanation for the multiplicity of adipokinetic hormones, and for the variation in their adipokinetic potencies in different species, may eventually be found in the variety of situations in which FFA mobilization occurs. This process, which makes the energy-rich fatty acids stored within adipose tissue rapidly available to the other organs of the body (35), appears to play a role in the adaptation of animals to several different conditions (105). The different circumstances under which FFA mobilization is known or may be suspected to occur, and the nature of the supporting evidence, are as follows:

(*a*) *Starvation.* The concentration of FFA in the plasma increases during fasting in all species so far examined (man [11, 12], monkey [44, 102], dog [45], rat [43], rabbit[10], and guinea pig[10]). Slices of adipose tissue from fasting rats produce FFA at a more rapid rate than those from the fed rat (16).

(*b*) *Growth.* Greenbaum showed (106) that when growth was induced in mature rats by the chronic administration of growth hormone, a progressive reduction in the lipid content of the body occurred. The isolated epididymal adipose tissue of young, rapidly growing rats releases FFA four times more rapidly than that of mature rats (107).

(*c*) *Exposure to Cold.* The plasma FFA concentration increases 175% in the rat within 4 hr after the animal is placed in an environment at 4° (108). Within 20 min after exposure to 4°, the rat's adipose tissue exhibits a 6- to 8-fold increase in the rate of production of FFA *in vitro* (63).

(*d*) *Reproduction.* The serum FFA concentration (109) and serum triglyceride concentration (110) increase progressively during pregnancy in the woman. Ovulation in the pigeon and dove is associated with a 4- to 7-fold increase in the concentration of total lipids in the plasma as compared to the concentration before or after ovulation (50).

[11] The mechanism by which reduced availability of insulin or glucose to the fat cell stimulates FFA mobilization appears to be different from that by which the adipokinetic hormones produce this effect. This subject is considered in detail in recent reviews (26–29).

(*e*) *Migration.* Preceding migration in certain species of bird, large amounts of subcutaneous and intraperitoneal fat are deposited (111, 112). During migration, extensive mobilization of the fat depots occurs (113).

(*f*) *Hibernation.* Most hibernating animals deposit considerable amounts of fat during the months preceding hibernation (114). During hibernation, a marked reduction in oxygen consumption occurs and the respiratory quotient declines to 0.7. Arousal from hibernation is associated with a rapid increase in the rate of oxygen consumption. The rate of mobilization of FFA from adipose tissue during these periods has not yet been measured.

(*g*) *Fear.* The plasma FFA concentration of men was found to increase by 30–130% within 45 min after anxiety-producing stimuli (115, 116).

Further studies will be required to establish which of the several possible endocrine mechanisms (i.e., reduced secretion of insulin, or increased secretion of ACTH, TSH, other pituitary adipokinetic polypeptides, growth hormone, epinephrine, or norepinephrine) is operative in each species in each of the above situations. Observations suggesting a role of hormones with adipokinetic activity in certain of these situations may be mentioned. Exposure to cold evokes in the rat increased secretion of TSH, ACTH, and catechol amines (108, 117–120). Increased secretion of one or more of these hormones also occurs in the guinea pig, rabbit, and dog after exposure to cold (119, 120). Species differences in the endocrine response to cold are suspected (114, 121) but have not yet been systematically studied. Increase in the circulating level of thyroid hormone is found in the pregnant woman (122). Alterations in pituitary morphology and function take place in the bird in association with migration (111). It is possible to visualize how mobilization of FFA could be coupled to other endocrine effects appropriate to a specific situation, if the pituitary or adrenergic hormone secreted in response to the specific stimulus possessed, in addition to its other biologic properties, adipokinetic activity in the species involved. Evaluation of this concept requires simultaneous observations of several different aspects of endocrine function and the rate of FFA mobilization in different species during starvation, exposure to cold, reproduction, etc. Such information is not available at present.

4. *Other Biologic Actions of Adipokinetic Pituitary Polypeptides.* The adipokinetic pituitary polypeptides ACTH, TSH, arginine vasopressin, α-MSH, and β-MSH act upon other target organs in addition to adipose tissue; e.g., adrenal cortex, thyroid, renal

tubule, and melanocyte. The more recently identified "peptide I" has been shown by Krayer and coworkers (123) to accelerate the rate of contraction of the isolated canine heart, as do ACTH, α-MSH, and β-MSH (123, 124). Further studies may bring to light still other biologic properties of these polypeptides. Of special interest is the acute (i.e., within 2 hr after subcutaneous injection) calorigenic effect of certain adipokinetic pituitary polypeptides. In 1938, O'Donovan and Collip reported (125) that subcutaneous injection of pituitary extracts causes an acute increase in the oxygen consumption of the rabbit. Intermediate lobe preparations (presumably rich in the MSH's) were especially potent in this respect. ACTH has been observed to have an acute calorigenic effect in the mouse (126) and guinea pig.[10] In all three of these instances, calorigenic activity is associated with adipokinetic activity. Nevertheless, the increase in oxygen consumption is not necessarily the result of oxidation of newly mobilized FFA, since fraction H has been found to have an adipokinetic but not a calorigenic effect in the rabbit (20). Another example of the dissociation of the adipokinetic and calorigenic activities is apparent in the effects of epinephrine in the rabbit, in which species this hormone possesses the latter (127) but not the former (25) activity. Further studies of this biologic property of the adipokinetic polypeptide and amine hormones, and of its mechanism, may contribute to our understanding of the role of the endocrine system (120) in the regulation of body temperature and in adaptation to cold.

In susceptible species, certain of the adipokinetic pituitary polypeptides, like the adrenergic amines, thus accelerate not only the mobilization of FFA from adipose tissue, but also the heart rate and the rate of oxygen consumption of the whole animal. The implication arises that in certain species polypeptide hormones of the pituitary gland may influence processes which, in other species, are regulated exclusively or principally by the catechol amines. In order to evaluate this possibility, the pharmacologic effects of the adipokinetic pituitary polypeptides and of the catechol amines must be compared in a more comprehensive manner, and in a larger series of vertebrate species, than has yet been undertaken. Primitive vertebrate species may be of special importance for clarification of the phylogenetic aspects of this subject, as they have proved to be in studies of the comparative endocrinology of antidiuretic and oxytocic peptides (128).

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