J. Lipid Research, July 1963
Volume 4, Number 3

Concentration of norepinephrine, serotonin, and histamine, and of amine-metabolizing enzymes in mammalian adipose tissue*†

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[Manuscript received March 11, 1963; accepted April 1, 1963.]

SUMMARY

The norepinephrine content of adipose tissue is shown to be very different in various animal species and different sites of origin, ranging from 0.03–1.4 μg/g. Adipose tissue also contains considerable amounts of serotonin (0.01–1.04 μg/g) and histamine (0.1–13.6 μg/g). Changes in the norepinephrine content of adipose tissue after the injection of either reserpine analogues or monoamine oxidase inhibitors followed a pattern similar to that found in the heart and brain, indicating that the storage mechanism in these organs is basically the same. In contrast to norepinephrine, serotonin in adipose tissue is rather resistant toward depletion by reserpine. Adipose tissue also contains monoamine oxidase and catechol-O-methyltransferase activity, which are usually highest in tissues also rich in norepinephrine.

There is good evidence that the sympathetic nervous system is involved in the mobilization of free fatty acids (FFA) from adipose tissue (1). Both norepinephrine and epinephrine have repeatedly been demonstrated to mobilize FFA in vivo and in vitro (1), possibly by activating a lipolytic system in adipose tissue (2). Mobilization of FFA by other means, such as sympathetic stimulation, stress, or the injection of corticotropin (ACTH), may very well be mediated by endogenous catecholamines in fat tissue, since it cannot be induced after the fat has been depleted of catecholamines by syrosingopine or reserpine. In rats, FFA mobilization following the administration of tyramine, which acts through liberation of endogenous norepinephrine, is completely abolished by pretreatment with syrosingopine (3); similarly, the increase of plasma FFA induced by ACTH has been reported to be blocked by pretreatment with reserpine[1] (4). On the other hand, FFA mobilization during cold stress was only reduced after pretreatment with syrosingopine, although the adipose tissue had been depleted by more than 90% (3).

Estimation of norepinephrine in adipose tissue of rats has been reported by several groups of authors (4–6). While there is fairly good agreement on the norepinephrine content of white epididymal fat tissue, ranging from 0.05–0.12 μg/g, the data reported for brown interscapular fat cover the relatively wide range from 0.15–1.6 μg/g. Therefore, it seemed desirable to reexamine the norepinephrine content of rat adipose tissue. In the present studies, norepinephrine concentration was determined in adipose tissue of various animal species and of different sites of origin. Enzyme activities connected with the metabolism of catecholamines were measured. In addition, the presence of serotonin and histamine in adipose tissue was demonstrated.

MATERIALS AND METHODS

Male Wistar rats (180–220 g), male albino mice (18–22 g), and male guinea pigs (300–500 g) were used and kept on standard pellet diet, obtained from the Hannover'sche Tierzuchtanstalt (Hannover, Germany). The animals were killed by decapitation and exsan-

* Supported by a grant of the Deutsche Forschungsgemeinschaft (We 272).

† A preliminary report was given at the Spring meeting of the Deutsche Pharmakologische Gesellschaft (April 29–May 1, 1963.)

[1] While preparing the manuscript, a paper by Edmonson and Goodman came to our attention; these authors were unable to confirm this finding (29).

guinated. Heart, brain, small intestine, interscapular and epididymal fat, and, in guinea pigs, also perirenal and lumbar fat, were rapidly excised, weighed, and stored at −18° until the determination of the amine content. Care was taken to dissect interscapular fat free from surrounding white fat and striated muscle.

For the determination of *norepinephrine*, 1–2 g tissue samples (4–6 g in the case of white fat) were suspended in 15–25 ml of ice-cold 5% trichloroacetic acid (TCA), homogenized with an "Ultraturrax" (Janke & Kunkel, Staufen, Germany) homogenizer, and centrifuged for 15 min at 1,100 × *g*. The sediment was resuspended in 10 ml TCA and recentrifuged, and the combined supernatant fluids were filtered. The final volume was brought to 30 ml with distilled water. After adding 350 mg of disodium-ethylene-diaminetetraacetic acid (Titriplex, Merck, Darmstadt), adsorption on Al_2O_3 was performed according to the method of Euler and Orwén (7). Norepinephrine was eluted with 4.0 ml of 0.25 N HCl and fluorimetrically determined according to the method of Shore and Olin (8). The fluorimetric determination of *serotonin* followed the method of Bogdanski et al. (9); *histamine* was measured according to the method of Shore, Burkhalter, and Cohn (10), using an Aminco-Bowman spectrophotofluorimeter. All given wavelengths are uncalibrated instrument values. Known amounts of the respective amine were added to a TCA solution and carried through the whole extraction procedure (internal standards). Accordingly, all figures for the amines (expressed as μg/g wet weight) were corrected for the loss during the extraction procedure. Recovery amounted to 62 ± 1.4% for norepinephrine, 91 ± 2.2% for serotonin, and 73 ± 2.6% for histamine.

Bioassays of the extracted norepinephrine were made by recording the blood pressure of rats under urethane anesthesia (1.5 g/kg s.c.) and ganglionic blockade (1.0 mg/kg chlorisondamine, Ecolid, Ciba, Basel).

Monoamine oxidase was assayed according to the method of Sjoerdsma et al. (11). Homogenates containing 25–500 mg adipose tissue/ml distilled water were incubated with 30 μg serotonin using a Dubnoff shaker for 30 min at 37.5° (pH was adjusted to 7.4 with 0.5 ml 0.5 M phosphate buffer; final volume was 3.0 ml). Monoamine oxidase activity is expressed as μg/g/hr serotonin deaminated. Possible losses of serotonin not due to enzymatic degradation were checked for by preincubating homogenates with a potent inhibitor of monoamine oxidase (100 μg/ml benzyl-methyl-propynylamine, MO 911, Eutonyl, Abbott Labs., North Chicago, Ill.) for 15 min before the addition of serotonin. The serotonin content of the incubation mixtures was determined according to the method of Bogdanski et al. (9).

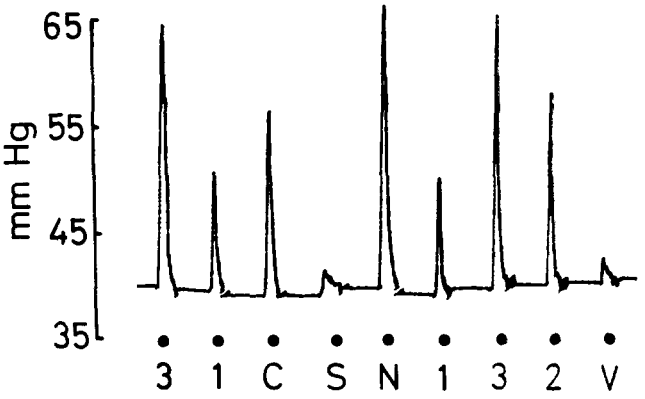

FIG. 1. Identity of pressor material extracted from interscapular fat of rats with norepinephrine. Blood pressure of the rat (210 g), urethane anesthesia, ganglionic blockade (see Methods). Intravenous injection of 0.1 ml of the neutralized extracts of interscapular fat of rats corresponding to 400 mg/ml. Abbreviations: 1, 2, and 3 = 0.1 ml of the neutralized internal standards corresponding to 0.025, 0.05, and 0.075 μg norepinephrine; C = controls; N = 16 hr after 50 mg/kg s.c. nialamide; S = 16 hr after 0.5 mg/kg s.c. syrosingopine; V = volume effect of 0.1 ml of the neutralized blank.

Catechol-O-methyltransferase was assayed by measuring the rate of formation of normetanephrine from norepinephrine (12). Various tissues were homogenized in 2 vol of isotonic KCl and centrifuged for 30 min at 30,000 × *g*. The supernatant fluid was used as enzyme preparation. Two milliliters of supernatant fluid corresponding to 50–900 mg tissue, 0.5 ml phosphate buffer (0.5 M, pH 7.9), and 0.1 ml 0.3 M $MgCl_2$ were preincubated for 10 min with 0.1 ml 0.008 M benzyl-methyl-propynylamine to block residual monoamine oxidase. Following the addition of 0.2 ml 0.005 M norepinephrine and 0.1 ml 0.004 M S-adenosylmethionine, incubation was carried out for 30 min at 37° in a Dubnoff shaker. To stop the reaction, 4.0 ml of borate buffer (0.5 M, pH 10.2) and 80 mg of solid sodium bicarbonate were added, and the mixture was shaken for 15 min to destroy the excess of norepinephrine. The incubation mixtures were transferred to stoppered centrifuge tubes containing 40 ml *n*-butanol and 3 g of solid NaCl. After shaking for 30 min and centrifuging, 15 ml of the butanol phase was transferred to centrifuge tubes containing 30 ml of *n*-heptane and 3.0 ml of 0.1 N HCl and shaken for 10 min. Two milliliters of the aqueous phase were removed and fluorimetrically assayed for normetanephrine (12). Internal standards were always carried through the whole procedure. Recovery amounted to 65–70%. Enzyme activity is expressed as μg/g/hr normetanephrine formed.

TABLE 1. NOREPINEPHRINE CONTENT (μg/g) OF ADIPOSE TISSUES AND HEART IN FOUR SPECIES

Animals		Adipose Tissue				
Species	No.	Interscapular	Epididymal	Perirenal	Lumbar*	Heart
Rat	61	1.40 ± 0.50†	0.042 ± 0.006	—	—	0.97 ± 0.026
Mouse	30	0.77 ± 0.042	—	—	—	0.73 ± 0.020
Guinea Pig	36	0.31 ± 0.014	0.11 ± 0.017	0.56 ± 0.054	0.30 ± 0.016	2.49 ± 0.13
Rabbit	2	0.033; 0.045	0.014; 0.021	—	—	2.18; 2.46

* Located intraabdominally on the musc. psoas.
† ± Standard error.

RESULTS

Identification of Norepinephrine Extracted from Adipose Tissue. The activation and fluorescence spectra of authentic norepinephrine and norepinephrine extracted from interscapular fat of rats had the same characteristics with activation maxima at 400 mμ and fluorescence maxima at 520 mμ. Using a bioassay technique, the extracted norepinephrine elicited a pressor response similar to that elicited by synthetic norepinephrine (Fig. 1). The fluorescent and pressor material extracted from adipose tissue was depleted by pretreatment with syrosingopine (S) and had accumulated following the injection of nialamide (N). The results of the biochemical and pharmacological methods were in good agreement.

Norepinephrine Content of Adipose Tissue. The results are summarized in Table 1. The highest amount of norepinephrine in adipose tissue was found in the interscapular brown fat of rats (1.4 μg/g), while approximately one-thirtieth as much was found in the white epididymal fat of the same species (0.04 μg/g). The brown interscapular fat of mice contained considerably less norepinephrine (0.77 μg/g) than the corresponding fat tissue of rats. Still smaller amounts of norepinephrine were extracted from the interscapular fat of guinea pigs (0.31 μg/g) and rabbits (0.033 μg/g). In guinea pigs, the highest norepinephrine content was found in perirenal fat (0.56 μg/g), while the adjacent lumbar fat contained only about half as much (0.30 μg/g).

It should be noted that the interscapular fat of the various animal species examined is grossly different in appearance. In rats and mice, the brown butterfly-shaped fat body can be clearly separated from the surrounding tissues, while in guinea pigs, the pale-yellow interscapular fat is sometimes difficult to distinguish from white fat. Rabbits and cats have very little interscapular fat.

Depletion of Norepinephrine in Adipose Tissue. The rauwolfia alkaloid reserpine is well known to deplete tissues of catecholamines by impairing the capacity of the cell to store amines. Syrosingopine, a semisynthetic analogue of reserpine, has essentially the same properties. At a certain dose range, however, it is ineffective in releasing norepinephrine from the central nervous system, while peripheral tissues (e.g., heart) are depleted of catecholamines (13). The rate of deple-

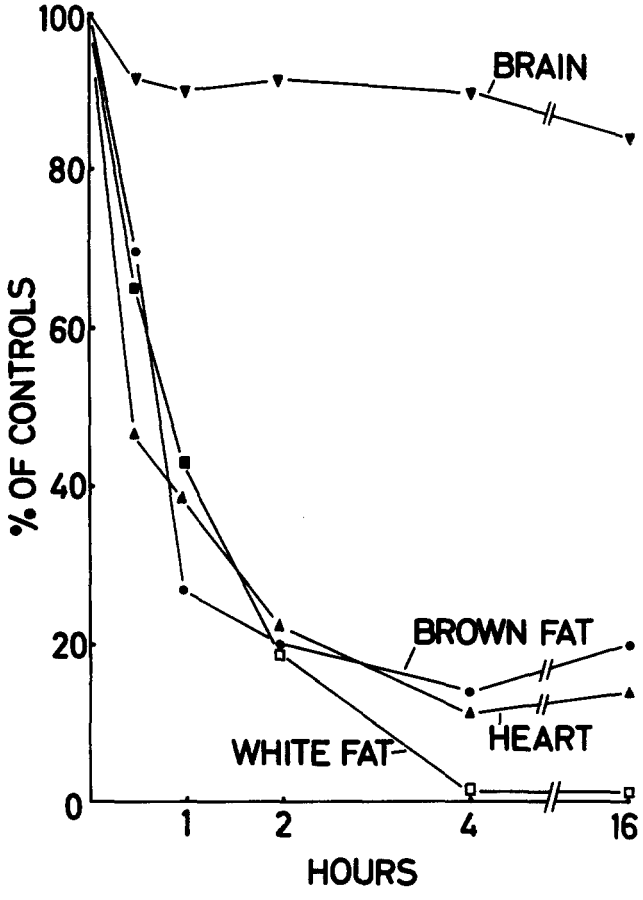

FIG. 2. Effect of syrosingopine on the norepinephrine content of adipose tissue, heart, and brain of rats. Each point represents the average of four animals. Groups were killed 30 min, 1, 2, 4, and 16 hr after 0.5 mg/kg s.c. syrosingopine. Control values at zero time: brown fat (interscapular) 1.23 μg/g, white fat (epididymal) 0.06 μg/g, heart 0.94 μg/g, brain 0.40 μg/g. Open symbols indicate values below the sensitivity of the method.

tion following the injection of syrosingopine is identical for interscapular brown fat, epididymal white fat, and heart, while brain norepinephrine is not appreciably lowered at any time within 20 hr (Fig. 2). The disappearance of norepinephrine from adipose tissue can also be demonstrated by bioassay (Fig. 1).

Serotonin and Histamine in Adipose Tissue. Adipose tissue also contains considerable amounts of serotonin and histamine. The activation and fluorescence spectra of these amines extracted from interscapular fat of rats had the same characteristics as authentic serotonin and histamine (Fig. 3). In rats and mice, interscapular fat contains 5–10 times more serotonin and histamine than the white epididymal fat (Table 2). Adipose tissue of guinea pigs also contains histamine but only traces of serotonin. While in rats and mice, the serotonin and histamine content of interscapular fat proved to be quite uniform, as indicated by the small standard error of the mean values, the amine content found in epididymal fat covered a relatively wide range (serotonin: 0.08–0.19 μg/g; histamine 1.38–5.8 μg/g).

Following a single injection of reserpine (2.5 mg/kg s.c.), the serotonin content of the brain declined by 70%, while the serotonin content of adipose tissue was lowered only by 40%. Repeated treatment with reserpine was necessary to lower the serotonin content of adipose tissue by more than 60% (Table 3, Fig. 3A). The storage mechanism of serotonin in the small intestine proved to be still less sensitive to repeated treatment with reserpine, which lowered the serotonin content only by 40%.

Compound 48/80 (Burroughs Wellcome Co, Tuckahoe, N.Y.) has been shown to decrease the histamine content of tissues in vivo (14). In rats treated for several days with increasing doses of 48/80, the histamine content of adipose tissue was reduced by more than 70%, while the histamine content of the heart was only lowered by 50% (Table 4, Fig. 3B).

Monoamine Oxidase in Adipose Tissue. Monoamine oxidase activity has recently been demonstrated to be present in adipose tissue (6). The monoamine oxidase activities of adipose tissues of various species and sites of origin are shown in Table 5. In summary, it can be stated that in any given species the highest activities are usually found in tissues that are also rich in norepinephrine. Since monoamine oxidase inhibition leads to a pronounced increase in tissue amines (15), the action of nialamide (Niamid, Pfizer, Karlsruhe), an irreversible monoamine oxidase inhibitor, on the norepinephrine content of fat and other tissues was studied. Both the biochemical and the biological determination (Fig. 1) revealed that adipose tissue norepinephrine behaves like catecholamines in other organs. Plotting the time after the injection of nialamide against the level of norepinephrine found in interscapular and

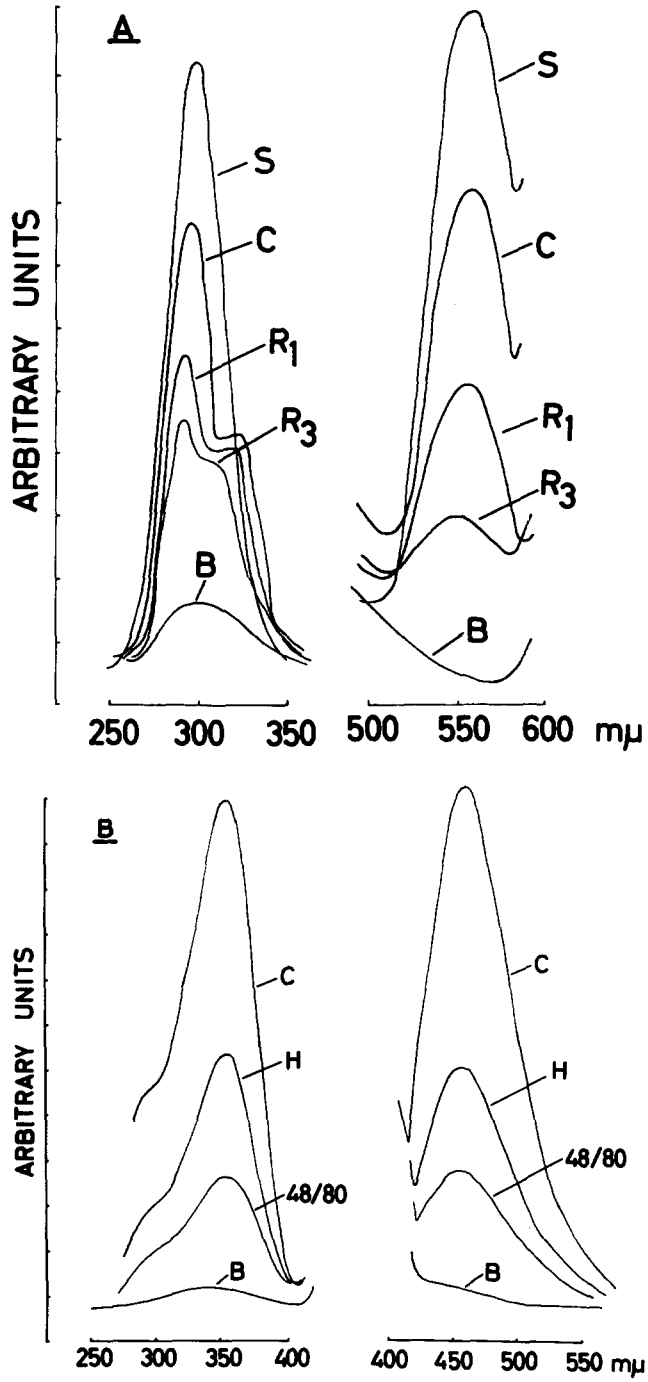

FIG. 3. Activation (left) and fluorescence (right) spectra of serotonin (A) and histamine (B). To obtain activation spectra, the fluorescence monochromator was set at 555 mμ for serotonin and at 455 mμ for histamine, and the spectra from the activating monochromator were scanned. To obtain fluorescence spectra, the activation monochromator was set at 295 mμ for serotonin and at 350 mμ for histamine, and the spectra from the fluorescence monochromator were scanned. *A:* Spectra of authentic serotonin (S), reagent blank (B), extract of interscapular fat of control rats (C) and of rats pretreated with 2.5 mg/kg reserpine once (R_1) or on three consecutive days (R_3). *B:* Spectra of authentic histamine (H), reagent blank (B), extract of interscapular fat of control rats (C) and of rats pretreated with 15 mg/kg of compound 48/80 (48/80). For details of pretreatment see Tables 3 and 4.

TABLE 2. SEROTONIN AND HISTAMINE CONTENT OF ADIPOSE TISSUE

Animals		Serotonin (μg/g) ± SE			Histamine (μg/g) ± SE	
Species	No.	Interscapular Fat	Epididymal Fat	No.	Interscapular Fat	Epididymal Fat
Rat	43	1.04 ± 0.054	0.154 ± 0.018	21	13.6 ± 0.70	2.54 ± 0.40
Mouse	10	0.12 ± 0.010	0.025	10	1.60 ± 0.20	0.10
Guinea Pig*	10	0.03 ± 0.020	0.015	5	0.71 ± 0.015	0.69

* In guinea pigs, lumbar fat contained 3.13 μg/g, perirenal fat 2.13 μg/g histamine (average of the pooled tissues from 5 animals); no serotonin was found in these tissues. All values are means ± standard error (SE).

TABLE 3. EFFECT OF RESERPINE ON THE SEROTONIN CONTENT OF ADIPOSE TISSUE, SMALL INTESTINE, AND BRAIN*

Animals									
Group	No.	Interscapular Fat		Epididymal Fat		Small Intestine		Brain	
		μg/g ± SE	%	μg/g ± SE	%	μg/g ± SE	%	μg/g ± SE	%
Controls	43	1.04 ± 0.054	100	0.154 ± 0.018	100	3.48 ± 0.35	100	0.44 ± 0.023	100
Reserpine 2.5 mg/kg	11	0.67 ± 0.06	64	0.093 ± 0.019	60	2.96 ± 0.15	85	0.13 ± 0.035	29
Reserpine 7.5 mg/kg	10	0.33 ± 0.065	32	0.070 ± 0.020	45	2.00 ± 0.26	58	0.04 ± 0.020	9

* Rats (180–220 g) were injected s.c. with 2.5 mg/kg reserpine once or on three successive days, receiving a total of 7.5 mg/kg. Twenty hours after the last injection, the animals were killed for the determination of serotonin (see Methods). All values are means ± standard error (SE).

TABELE EFFECT OF COMPOUND 48/80 ON THE HISTAMINE CONTENT OF ADIPOSE TISSUE AND HEART*

Animals							
Group	No.	Interscapular Fat		Epididymal Fat		Heart	
		μg/g ± SE	%	μg/g ± SE	%	μg/g ± SE	%
Controls	21	13.6 ± 0.70	100	2.54 ± 0.4	100	2.28 ± 0.14	100
Compound 48/80	8	2.94 ± 0.43	22	0.70 ± 0.2	28	1.12 ± 0.14	49

* Rats (200–230 g) were injected s.c. on four successive days with 1, 2, 4, and 8 mg/kg of compound 48/80, receiving a total of 15 mg/kg. Twenty hours after the last injection, the animals were killed for the determination of histamine (see Methods). All values are means ± standard error (SE).

TABLE 5. MONOAMINE OXIDASE ACTIVITY (μg/g/hr) IN ADIPOSE TISSUE AND HEART OF VARIOUS SPECIES*

Animals		Adipose Tissue				
Species	No.	Interscapular	Epididymal	Perirenal	Lumbar	Heart
Rat	16	329	91	—	—	2600
Mouse	18	262	—	—	—	116
Guinea Pig	12	242	158	270	176	508

* Expressed as rate of oxidation of added serotonin substrate (see Methods). The data of this table represent the mean of at least three experiments wherein the tissues of 2–5 animals were pooled for the enzyme preparations.

epididymal fat of rats, a prompt and continuous increase over the whole time range can be observed that follows a pattern similar to that found in the heart and the brain (Fig. 4). Harmaline, being a reversible short-acting monoamine oxidase inhibitor itself (16), has been shown to protect monoamine oxidase against inhibition by irreversible inhibitors, if given prior to the latter (17, 18), and to prevent the rise in tissue amines (19, 20). As shown in Table 6, pretreatment with harmaline also prevents the blockade of monoamine oxidase and the increase of norepinephrine in adipose tissue after the administration of nialamide, indicating that the accumulation of norepinephrine is a true reflection of monoamine oxidase inhibition.

Catechol-O-Methyltransferase in Adipose Tissue. Catechol-O-methyltransferase is widely distributed among a variety of species and tissues, being consistently highest in the liver, while heart tissue contains only

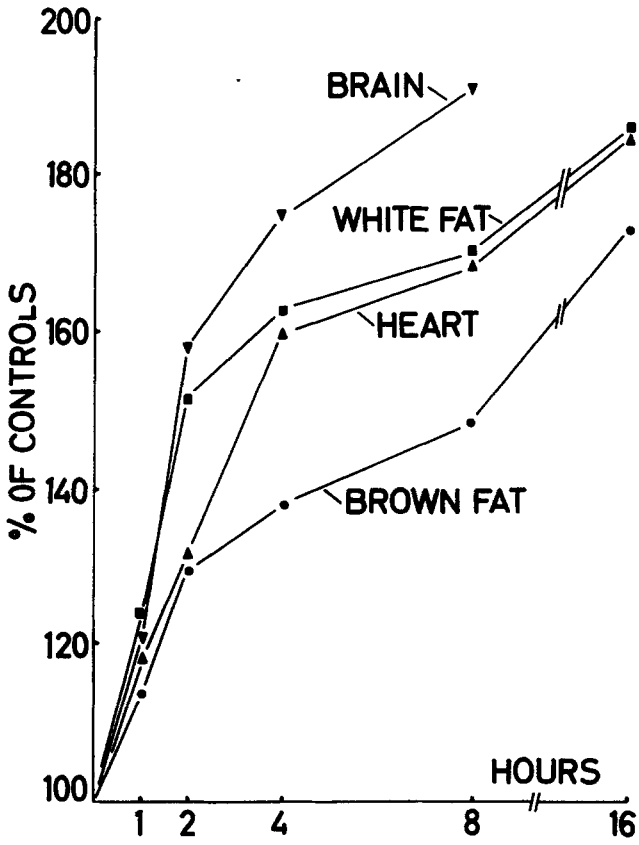

FIG. 4. Effect of nialamide on the norepinephrine content of adipose tissue, heart, and brain of rats. Each point represents the average of four animals. Groups were killed 1, 2, 4, 8, and 16 hr after 100 mg/kg s.c. nialamide. Control values at zero time: brown fat (interscapular) 1.44 μg/g, white fat (epididymal) 0.034 μg/g, heart 0.85 μg/g, and brain 0.38 μg/g.

TABLE 6. PREVENTION OF NIALAMIDE-INDUCED MONOAMINE OXIDASE INHIBITION AND NOREPINEPHRINE INCREASE IN ADIPOSE TISSUE BY PRETREATMENT WITH HARMALINE*

	Adipose Tissue			
	Interscapular		Epididymal	
Group	NE	MAO	NE	MAO
Controls	1.71	290	0.08	88
Nialamide	2.87	64	0.12	16
Harmaline	1.95	220	0.08	74
Harmaline + Nialamide	1.73	230	0.07	76

* Nialamide (50 mg/kg s.c.) was injected to rats 18 hr before decapitation, harmaline 1 hr prior to nialamide. Controls received saline. Norepinephrine (NE) content given as μg/g, monoamine oxidase (MAO) activity as μg/g/hr serotonin deaminated. Each value represents the average of four animals.

little activity (21). As can be seen from Table 7, the norepinephrine-rich interscapular fat of rats contains considerably more activity than the heart. In mice, catechol-O-methyltransferase activities in interscapular fat and heart were approximately the same. Under our experimental conditions, we were unable to demonstrate catechol-O-methyltransferase activity either in the white fat of rats and mice or in the heart and adipose tissue of guinea pigs. It should be noted, however, that, in our hands, the enzyme activities, e.g., of rat heart and liver, were considerably smaller than those reported by Axelrod and Tomchick (12).

DISCUSSION

Large amounts of norepinephrine can be isolated from adipose tissue, but the norepinephrine content is not only different in various species but also largely depends upon the site of origin of the fat tissue. In rats, the highest levels were found in the interscapular fat bodies, exceeding those of epididymal fat 30- to 40-fold. In guinea pigs, the norepinephrine content of interscapular, lumbar, and perirenal adipose tissue was of the same order of magnitude. The norepinephrine values found in the adipose tissue of rats and mice are in good agreement with those of Sidman et al. (5), though they differ markedly from the data of Paoletti et al. (4).

Comparing the norepinephrine content of adipose tissue with that of other organs (e.g., heart), no obvious correlation can be seen. In rats, significantly higher amounts were found in the interscapular fat than in the heart, while the reverse holds true in guinea pigs and rabbits; in mice, interscapular fat and heart contain approximately the same amount of norepinephrine.

Norepinephrine in adipose tissue responds to pharmacological procedures in much the same way as it does in other organs. Syrosingopine, an analogue of the rauwolfia alkaloid reserpine, depleted adipose tissue and heart norepinephrine at the same rate, indicating that the storage mechanism of norepinephrine in the two tissues is similar; on the other hand, pretreatment with monoamine oxidase inhibitors invariably caused a significant increase in fat tissue norepinephrine as well as in the heart or the brain.

The localization of the catecholamines within adipose tissue has not, to our knowledge, been studied. It is conceivable, however, that the norepinephrine is morphologically associated with the rich nerve supply of adipose tissue (22); this has recently been shown for the pineal gland, another organ rich in both sympathetic innervation and norepinephrine (23). The physiological significance of these large amounts of norepinephrine in adipose tissue is essentially unknown. There is accumulating evidence that the catecholamines are involved in the mobilization of free fatty acids. This

TABLE 7. CATECHOL-O-METHYLTRANSFERASE ACTIVITY (μg/g/hr) IN ADIPOSE TISSUE, HEART, AND LIVER OF VARIOUS SPECIES*

Animals		Adipose Tissue			
Species	No.	Inter-scapular	Epi-didymal	Heart	Liver
Rat	25	15.2	0.0	1.9	504
Mouse	30	6.2	0.0	7.5	286
Guinea Pig	8	0.0	0.0	0.0	47

* Expressed as rate of normetanephrine-formation (see Methods). The data of this table represent the mean of at least three experiments wherein the tissues of 2–5 animals were pooled for the enzyme preparations.

FFA mobilization has been shown to occur in vivo in subcutaneous fat (24), but there are no data as to what extent the brown adipose tissue contributes to the release of FFA in vivo. Nevertheless, depletion of norepinephrine in adipose tissue renders sympathetic stimulation as well as cold stress less effective in mobilizing FFA (3, 6). On the other hand, an increase in adipose tissue norepinephrine by pretreatment with monoamine oxidase inhibitors potentiates the release of FFA during cold stress, although other stress parameters induced by ACTH hypersecretion are unchanged or even depressed (6).

Adipose tissue also contains considerable amounts of serotonin and histamine. Although the physiological significance of these amines is still obscure, it should be noted that the distribution of serotonin and histamine in adipose tissue is similar to that found for norepinephrine; in brown interscapular fat of rats and mice, the levels of serotonin and histamine proved to be 5–10 times higher than in white epididymal fat. Additionally, our experiments with reserpine indicate that the storage mechanism of serotonin in adipose tissue is less sensitive to rauwolfia alkaloids than the storage mechanism of norepinephrine; in the brain, serotonin and norepinephrine are depleted by reserpine at the same rate.

Like other organs rich in amines, adipose tissue possesses enzymes for their synthesis and inactivation. While preliminary experiments have shown the dopa-/5-hydroxytryptophane decarboxylase activity to be very small (6), appreciable monoamine oxidase activity was found in adipose tissue, particularly in the interscapular fat where norepinephrine and serotonin levels were also high. This is in agreement with a histochemical observation of Menschik (25), as well as with the finding that brown adipose tissue is extremely rich in mitochondria, a particulate cell fraction usually associated with most of the monoamine oxidase activity (26, 27). Brown interscapular fat of rats also contains catechol-O-methyltransferase activity, which was found to be higher than that of the heart while it is absent in white epididymal adipose tissue.

Thus, brown fat exceeds white fat in the content not only of biogenic amines but also of enzymes involved in their metabolism, which may indicate a relatively higher amine turnover in brown adipose tissue. These findings match the general picture of brown fat being a metabolically highly active organ with great respiratory activity (28). The physiological function of brown fat, however, remains obscure.

We are indebted to Mrs. Erika Dargatz and Miss Reinhild Thiel for their skillful technical assistance.

Drugs mentioned in the text were generously supplied by the respective manufacturers.

We are particularly grateful to Prof. G. Kroneberg (Farbenfabriken Bayer, Leverkusen, Germany) for his generous aid in obtaining animals.

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