Tissue Weights of the Rat. I. Normal Values Determined By Dissection and Chemical Methods. (22186)

W. O. Caster, Judith Poncelet, Ada B. Simon, and W. D. Armstrong.
Department of Physiological Chemistry, University of Minnesota, Minneapolis.

Change in body weight, or more particularly tissue weights, is an important and physiologically significant datum in many experimental situations. This paper will describe practical methods for obtaining and expressing tissue weights required for this type of study. These procedures result from experience gained in quantitative dissection of some 300 rats (2-3000 tissue weights) in the course of a 4-year study of alterations in tissue metabolism associated with radiation sickness. They are designed to provide a statistically stable base-line for estimating changes in weight of each major tissue component of the body. The weight of such organs as liver, kidneys, spleen, testes, etc., can easily be obtained by direct dissection, and thus require little special discussion. Unfortunately their total weight constitutes only a minor fraction of total body weight and frequently contributes in a trivial way to a total body weight change. The major portion of body weight lies in the musculoskeletal system. Because of extensive interconenction between muscle, skeleton, marrow and connective tissue, this fraction also presents one of the greatest problems in quantitative separation of different components. For purposes of description, we have arbitrarily divided the musculoskeletal system into 2 major parts: (a) total muscle mass, and (b) non-muscle components which are grouped under the general heading of supportive tissue. A more complete description of the various components of these two major parts will be given as we proceed.

The method for determining how much of the total musculoskeletal weight can be ascribed to each of these two categories depends upon the 2 observations that: (a) some 70% of the muscle mass of the rat can be removed from the supportive tissue in about 20 minutes, and (b) there are sufficiently large differences between the chemical compositions of supportive tissue and muscle so that it is possible, by direct chemical means, to estimate the amount of muscle still adhering to the supportive tissue at the end of the partial dissection. Chief among the chemical differences between these tissues are the differences in per cent water, per cent ash, and the differences in actomyosin, desoxyribonucleic acid and creatine concentrations. This approach has not only the advantages of speed and ease, but also yields such information as the fresh weight (and per cent water) of supportive tissue fractions and the weight of the total marrow mass which is difficult to obtain by any other method. For further discussions this partially dissected sample, consisting on a fresh weight basis of nearly equal weights of muscle and supportive tissue, will be termed the "stripped carcass." The ideal substance to use in estimating the relative weights of muscle and supportive tissue in the stripped carcass, would be one which is constant in all muscle, absent in skeleton and connective tissue, and is easily and accurately determinable. Empirically, the determination of actomyosin or the per cent of ash have been found most useful for this purpose. Where other analyses are also desired on this same sample the ash procedure is the more convenient. This combination dissection-chemical approach was also used in estimating the total amount of marrow in the skeleton. The useful chemical data in this case were the desoxyribonucleic acid and hemin contents.

Chemical methodology. The reagents and general pattern of procedure for determining actomyosin are those described by Benson, Hallaway and Freier(1). Quickly dissect, weigh and freeze the sample for storage. Chop the frozen sample, place in a water cooled Waring blendor cup with 3½ ml of Webers solution (0.6M KCl in bicarbonate buffer) per gram of sample, and blend 8 minutes at reduced speed (furnish 40-60 volts AC to the Waring blendor motor). Wash into a beaker

TABLE I. Tissue Weights Expressed in Different Ways.

Tissue	As % of body wt	As % of Wc* Mean	As % of Wc* σ	Data of Column 2 expressed on a: Fat-free basis	Dry, fat-free basis
GI content†	5.85	—	—	—	—
Depot fat‡	7.08	—	—	—	—
Muscle	45.5	52.3	1.5	52.9	42.1
Supportive	7.68	8.82	.52	8.98	19.24
Skin	18.0	20.7	1.6	19.2	26.6
Liver	4.15	4.77	.53	4.96	4.39
Lung	.56	.64	.10	.66	.43
Heart	.289	.332	.030	.345	.238
Kidneys	.76	.87	.15	.89	.69
Testes	.94	1.08	.13	1.13	.45
Spleen	.213	.245	.034	.256	.187
Brain	.550	.632	.046	.614	.300
Stomach	.502	.577	.038	.581	.435
Small intestine	1.93	2.22	.27	2.21	1.53
Large intestine	.89	1.02	.11	1.00	.63
Thymus	.125	.143	.025	.146	.095
Blood§	4.95	5.68	—	6.04	3.88

* Body wt minus wt of the gastrointestinal contents and depot fat.

† Fresh wt of material in gastrointestinal tract (on *ad lib.* diet of Purina Chow).

‡ Largely adipose tissue, though a small amount of miscellaneous tissue (bladder, etc.) was included.

§ Including fluid loss during dissection (by difference). For further details on blood volume and distribution see Caster, Simon, and Armstrong(8).

with an equal volume of 0.6M KCl, add 10 μl of 10% ATP per gram of stripped carcass, cover, and allow to stand in a refrigerator for 24 hours. Stir vigorously, transfer 20 ml aliquot(s) into centrifuge tube(s), add 20 ml of 0.6M KCl, centrifuge at 600 g for 10 minutes, transfer supernatant and recentrifuge for 15 minutes. Measure the viscosity of the supernatant before and after the addition of 20 μl of 5% ATP. The viscosity decrease observed for the stripped carcass is compared with that found for a sample of thigh muscle from the same animal, and the final result expressed as the per cent muscle estimated to be present in the stripped carcass (about 50%). The use of the per cent ash in the stripped carcass as a basis for estimation depends upon the fact that muscle contains only 4.58 $\pm$ .12% ash (on a dry, defatted basis) while the total supportive tissue was found in a separate series of dissections to contain 53.6 $\pm$ .7% (on a dry, defatted basis). The fresh weight figures could be used, but by expressing results on a dry-weight basis one eliminates errors due to evaporation loss and minimizes those resulting from physiological changes in the degree of hydration of the connective tissue. From these data it follows that for mixtures of muscle and supportive tissue the relation between composition and observed ash can be expressed as $X = 4.58 + .4898Y$, where $X = \%$ ash observed in the dry, defatted stripped carcass, and $Y = \%$ supportive tissue in the sample (expressed on a dry, fat-free basis). Desoxyribonucleic acid and hemin were determined in freshly extruded femoral marrow and in aliquots of completely dissected skeleton which had been dried, defatted and powdered in a Wiley mill to pass a 40-mesh screen. The DNA was hydrolyzed and extracted with 5% trichloroacetic acid at 85°C for 30 minutes, and the desoxyribose determined colorimetrically with Dische reagent(2). Since high concentrations of bone mineral interfere with maximal color development, a series of standards were analyzed in the presence of bone mineral so as to allow correction for this factor. For the determination of skeletal hemin, other aliquots were extracted with 0.1N NaOH, and the iron porphyrin determined by the pyridine hemochromogen method of Remington (3). This procedure is somewhat easier and more desirable because no empirical corrections are required.

Manner of expressing tissue weights. In

comparing and discussing the observed tissue weights of different animals, it would be convenient to express these data in relative rather than in absolute terms. The most usual way of doing this is to express tissue weight as a per cent of total body weight. However, the total body weight contains two portions which may vary considerably from rat to rat and may change very rapidly under stress conditions; *i.e.*, the weights of the (a) body fat, and (b) gastrointestinal contents. It was found empirically that much more stable estimates were obtainable when results were expressed on the basis of a body weight corrected for these variables. The symbol, Wc, will hereafter be used to designate body weight minus the weight of body fat and gastrointestinal contents. The rats used in this study were male white rats (Holtzman Co., Madison, Wis.), 110 ± 10 days of age, weighing 338 ± 20 g. They were received at a weight of 250 to 280 g and raised to the final weight on a diet of Purina Chow in a 25°C room. Dissections were carried out under ether anesthesia. The tissue weight data are summarized in Table I.

Procedure. Skin and eviscerate the anesthetized rat, and remove the depot fat from the carcass. Remove the brain, spinal cord, and any extraneous tissue. Weigh the musculoskeletal remainder and remove a sample of thigh muscle for the determination of ash weight. Divide the musculoskeletal system so that one person can strip the muscle from the head and shoulder girdle, while the other person removes the muscle from the other portions. About 70% of the muscle mass can thus be removed from the supportive tissue in 20-25 minutes. After a few dozen dissections this procedure became highly repeatable, yielding a stripped carcass sample which weighed 18.18 ± .60% of Wc. After weighing, the stripped carcass was either: (a) analyzed for actomyosin or (b) dried and ashed. As described above, either of these procedures provides an estimate of the residual muscle on the sample. The total supportive tissue weight in this sample was 8.82 ± .52% of Wc (22 rats) on a fresh weight basis.

Components of supportive tissue. A separate series of total dissections (22 rats) indicated that the weight of the skeleton (with teeth and marrow, but freed of connective tissue) was 6.86 ± .33% of Wc, and that the teeth weighed 0.184% of Wc. Analysis of these skeletons for DNA and hemin followed by a comparison of these results with the DNA and hemin contents of freshly extruded femoral marrow led to an estimated total marrow weight of 0.40% of Wc. The weight of the connective tissue (present in the stripped carcass but removed during the total dissection of the skeleton) was estimated by difference to be (8.82 — 6.86 =) 1.96% of Wc. It should be noted that this does not constitute the total connective tissue of the body, but only that closely associated with the skeleton. All of these data are summarized in Table II, together with analytical data on the weights of water and fat present in each. All data are expressed as grams of weight for the average rat in this study (body weight = 337.7 g and Wc = 294.0 g). The composition of connective tissue was obtained by subtracting the composition of its muscle and skeleton components from the observed composition of the stripped carcass. The dry, fat-free skeleton (complete with teeth and marrow) was 4.33% of Wc, and its ash weight was 2.63% of Wc. These figures are in good agreement with those reported by Savchuck and Armstrong(4) for the 304 g rat. Their information on the individual bones may therefore be considered as an extension of Table II.

TABLE II. Summary of Supportive Tissue Components in the 338 g Rat.

Tissue	Wt in g			
	Fat	H_2O	Fat-free dry wt	Fresh wt
Total	1.07	9.37	15.49	25.93
Skeleton*	.83	6.59	12.73	20.15
Connective	.24	2.78	2.76	5.78
Teeth	.00	.11	.43	.54
Marrow	.01	.09	.81	1.17

* Including teeth and marrow.

Muscle mass. Subtracting the weight of the supportive tissue from the original weight of the musculoskeletal system sample, the total weight of muscle mass is obtained. This method of calculation, again, minimizes er-

TABLE III. Weight of Muscle Groups as % of Total Muscle Mass.

Muscle group	Mean	σ
Thigh	19.18	1.25
Legs	4.48	2.34
Abdominal	16.96	2.37
Shoulder	8.43	1.69
Face and head	4.06	.58
Back	22.44	1.73
Residual	20.62	6.24
Fat	3.81	.44

rors due to evaporation loss during dissection. The components of the total muscle mass were studied in a separate series of dissections. Table III lists the relative weight of the major muscle groups which comprise this total muscle mass. All results are expressed on a fat-free wet basis and the total fat associated with these muscles is given separately at the bottom of the table. The thigh muscles were obtained by making an incision around the dorsal border of the ilium and extending it medially and ventrally along the os coxae, being careful to avoid the psoas major and psoas minor muscles and the quadratus lumborum. Another incision was made around the knee joint, and the muscles between the two incisions were removed. Then the muscles of the leg from the knee joint on down were removed. The muscles of the foot were not taken. These muscles taken from both legs constitute the leg muscles referred to in Table III. A ventral incision was made in the midline and another incision was made dorsally, just lateral to the transverse processes of the abdominal vertebrae and extended as far caudally as the tip of the ilium and as far cranially as the false ribs. The muscles lying between these two incisions were removed. The psoas major, psoas minor, iliacus, and quadratus lumborum were cut from their origins and insertions. All of these muscles taken together were included in the abdominal muscle group. An incision was made around the scapula and the muscles were stripped from the scapula, brachium and antibrachium. These are referred to as the shoulder muscles. The head was severed at the level of the epistropheus bone and the mandible was removed so as to allow access to the muscles of mastication and the buccinator muscle. The superficial muscles of the head and skull were then removed. This group is referred to as the face and head muscles. The ribs were disarticulated and the intercostal muscles removed. To these were added the major portion of the muscle which could be removed from the trunk. This is defined in Table III as the back muscles. There still remained a certain amount of muscle on the skeleton which corresponded roughly with that present in the stripped carcass sample, and it is here referred to as the residual muscle. This is mainly composed of the anterior and lateral vertebral muscles and the serratus posterior muscles which are difficult to remove from the vertebrae, together with the muscles of the manus and the foot. As seen in Table III the thigh muscles constitute one of the largest of these muscle masses and is, on a percentage basis, also one of the most constant. From its ready availability it furnishes one of the most common sources for muscle samples for chemical analysis.

Other tissues. The items in Table I include two miscellaneous classifications: "body fat" and "blood." The subcutaneous and circumrenal fat constitute by far the largest part of "body fat," but also included in this category are such small tissues as, bladder, pancreas, spinal cord, eye balls, etc. Obviously any of these tissues could be removed from this miscellaneous classification and measured separately if one so desired. The item termed "blood" is even less well defined. At the end of the dissection it is quite usual to find that the sum of the recorded tissue weights equals 94-96% of the weight of the anesthetized animal as measured prior to dissection. By visual inspection it is quite obvious that a certain amount of blood and other fluids have been lost in the course of the dissection. The best evidence(5-8) indicates that some ⅔ of the blood volume is present in the large vessels and can be lost in the course of this procedure. Since the magnitude of this estimate corresponds so well with the observed 4-6% weight loss, we have arbitrarily termed it "blood." In a series of 22 normal rat dissections, a $-.83$ correlation ($P<.01$) was observed between variations in "blood" weight

and the variations in muscle mass weight when expressed as a per cent of Wc. We take this to indicate that another factor entering into this "blood" item is the loss of muscle water by evaporation during the course of the 20-25 minutes required to remove the major portion of the muscle mass. The constancy of the "blood" weight is therefore of interest as an index of the repeatability of the dissection procedure.

Results. Each figure in Table I represents the mean of 16 ± 5 independent dissections. In the first column, these data are expressed as a per cent of the total body weight of the intact rat. As indicated above, a more repeatable and usable result can be obtained if tissue weights are expressed as a per cent of Wc (body weight minus the weights of the contents of the gastrointestinal tract and the depot fat) as is done in the second column. In the third column are given the corresponding standard deviations. These help to indicate the magnitude of change in tissue weight resulting from stress conditions that one might expect to detect with confidence by this dissection-chemical method. When tissue weight comparisons are made on a completely fat-free and fat-free dry-weight basis the results seen in columns 4 and 5 are obtained.

Summary. A combined dissection and chemical approach to the problem of determining tissue weights has been described. It allows the exact determination of total muscle mass and supportive tissue weight in a fraction of the time required by the complete dissection approach. It also provides estimates of the total marrow weight and the fresh weight of the connective tissue, which are difficult to obtain in any other manner.

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Tissue Weights of the Rat. II. Changes Following 700 r Total Body X-Irradiation. (22187)

W. O. CASTER AND W. D. ARMSTRONG.
Department of Physiological Chemistry, University of Minnesota, Minneapolis.

In the first 4-6 days following total body x-irradiation with an LD_{50} dose of x-ray, the rat loses some 15% of its body weight. An exact description of this weight loss in terms of the weight changes of the individual tissues of the body is of considerable importance in providing a basis for any metabolic study of the nature of radiation damage.

Methods. Groups of 300-350 g male white rats (Holtzman Co., Madison, Wis.) were given 700 r of total body x-irradiation (140 kv, ¼ mm Cu, 1 mm Al) and sacrificed at one of a series of periods following irradiation. Unirradiated control animals for each of the different experimental periods were distributed within each group of experimental ani-