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A Simplified Method for Sampling Small Animal Carcasses for Analyses.* (28548)

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One of the greatest difficulties encountered in metabolic balance studies, using small laboratory animals as experimental subjects, is that of obtaining representative samples of the carcass of the animal for analyses.

Various methods have been employed to effect representative sampling. Such methods have recently been discussed by Mickelson and Anderson(1), who also presented a method of preparing carcasses for analysis. The carcass is autoclaved, slurried in a Waring blendor, and homogenized in a colloid mill. This method has the advantages of allowing the animal skin to be readily disintegrated (due to autoclaving), allows the analysis of multiple aliquots of the carcass for any and all components, produces a homogenate of very small particle size, and results in analytical values with very small standard deviations. However, quantitative transfer of the slurry from the blendor container to the colloid mill is necessary and the colloid mill is expensive. This led us to investigate a technique which would obviate a colloid mill.

The method described consists of autoclaving the carcass, blending it in a Waring blendor, and transferring aliquots of the slurry directly to tared weighing bottles. A large-bore pipette is used to sample the slurry while the blendor is in operation. This technique was used to prepare the carcasses of 5 weanling and 28 young-adult male albino rats for moisture, total nitrogen and energy determinations. Analytical values obtained are reported and discussed.

Materials and methods. The animals were 33 weanling male albino rats averaging 78 g in body weight. Twenty-eight of the animals were the subjects in a 53-day metabolic study involving 7 quadruplicates of animals and equalized daily feeding within each quadruplicate of 4 experimental isocaloric diets containing 8-57% protein (casein), 18.6-2% fat (lard and corn oil), and 65-21% carbohydrate (cerelose). At completion of the metabolic study the experimental animals

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were etherized, the gastrointestinal tract contents removed and discarded, and the carcasses were stored frozen until analyses could be completed. At time of initiation of the metabolic experiment, 5 male rats of similar size, age and parentage as the experimental animals were similarly killed, prepared and stored frozen for analyses. The composition of these animals was assumed to be representative of that of the animals at the start of the metabolic experiment and were used later to calculate the initial composition of the carcasses of the experimental animals under study.

Preparation of carcasses for analyses. Frozen carcasses were cut into cubes of approximately one-half to one inch size, placed in quart Mason jars, covered, and autoclaved at 15 lb pressure for 15 minutes. After cooling, the cut-up carcass was placed in a Waring blendor and a weighed amount of water was added. In our experience, where the empty carcasses ranged in weight from approximately 70-240 g and the water ranged from approximately 90-117% of the empty carcass weight, Waring blendor containers of 500 and 1000 ml proved adequate. In all cases the 500 ml container† was used. This container, with a screw cap, could, during the initial stage of blending, be inverted to prevent stalling of the electric motor by the lodging of the initially large carcass cubes against the cutters. As soon as the cutters were able to rotate freely, the mixture was blended 10 minutes. Then the screw cap was removed, and with the blendor still in operation and actively agitating all portions of the slurry, samples were withdrawn, using a large-bore plastic pipette,‡ and transferred rapidly to tared weighing bottles. Occasionally the volume of a homogenate was too great to allow sampling from the 500 ml container under the conditions described—*i.e.*, the container was too full to permit removal of the cap without overflow of the contents and/or all portions of the homogenate were not in constant agitation. In such cases, before sampling, the homogenate was transferred to and blended in a regular 1000 ml container.§ After sampling, the remainder of the homogenate was stored in wide-mouth, screw cap bottles at 4°C.

Analytical methods. Total nitrogen determination. Approximately 3 g of homogenate were pipetted into tared weighing bottles and quantitatively transferred to Kjeldahl flasks. Nitrogen was determined by the macro-Kjeldahl method(2).

Gross energy determination. Approximately 3 g of homogenate were transferred directly to a previously tared platinum dish placed inside a previously tared weighing bottle. After weighing, the platinum dish with contents was removed and dried under vacuum at 68°C for approximately 30 hours. The sample was then ignited in an Emerson adiabatic bomb calorimeter, as described by Swift and French(3). In most instances the platinum dish was weighed after drying and before ignition to arrive at dry matter content of the homogenate.

Moisture determination. Three g of homogenate were transferred to weighing bottles alone or to platinum dishes in weighing bottles (previous section), heated under vacuum at 68°C for approximately 30 hours, and weighed. Weight loss was taken to be moisture.

Conversion factor for calculations. Analytical values obtained for the homogenates (*i.e.*, total nitrogen (per cent), energy (calories/g) and dry matter (per cent) were converted to a fresh carcass basis by multiplying the former values by a factor obtained by dividing total weight of the homogenate by the empty body weight of the animal at slaughter.

Results. Analytical results for the 5 weanling rats killed at the beginning of experiment and for the 28 young-adult rats killed at the conclusion of 53 days on the experimental diets are presented in Table I. Values are given for the homogenates as analyzed and upon a fresh carcass basis. To be noted are the small standard deviations for analytical values, and especially the small coeffi-

† Catalog No. S-61662, E. H. Sargent & Co., Chicago, Ill.

‡ Catalog No. 29-256, Cambosco Scientific Co., Boston, Mass.

§ Catalog No. S-61654, E. H. Sargent & Co., Chicago, Ill.

TABLE I. Mean Analytical Values of Moisture, Protein and Energy with Measures of Variation Among Replicate Samples Within Animals.

Component		Mean analytical values (all animals)	Variation among samples within animals: Standard deviation Range*	Mean†	Coefficient of variation‡
Homogenate:	Moisture, %	80.36	.03- .42	.20	.25
	Protein, %	10.93	.03- .45	.13	1.19
	Energy, calories/g	1249.0	.4 - 7.0	4.1	.32
Fresh carcass:	Moisture, %	63.88	.05- .79	.37	.58
	Protein, %	20.07	.05- .34	.19	.95
	Energy, calories/g	2298.8	.8 -13.7	7.7	.34

* Standard deviation $= \left(\frac{\Sigma d^2}{n}\right)^{1/2}$, where Σd^2 = sum of deviations from the mean squared and n = number of analyses minus one (degrees of freedom).

† Mean standard deviation $= \left[\frac{n_1 (S.D._1)^2 + \ldots n_{33} (S.D._{33})^2}{n_1 + \ldots n_{33}}\right]^{1/2}$, where $n_1 - n_{33}$ are the degrees of freedom for each analysis and $S.D._1 - S.D._{33}$ are standard deviations for each analysis.

‡ Coefficient of variation $= \frac{\text{Mean standard deviation}}{\text{Mean analytical value}} \times 100$.

cients of variation. In general, 3 aliquots of each homogenate were used in arriving at each moisture determination, 4 for each protein determination and 2 for each energy determination. No limit was placed on agreement among moisture determinations as a criterion for acceptance as analytical results. For protein determinations, no analytical value was accepted in which the difference between the maximum value of the 4 aliquots exceeded the minimum value by more than 3.4% of the maximum value. For energy determinations, no analytical value based on analyses of 2 aliquots was accepted in which the larger value exceeded the smaller by more than one per cent of the larger. When 3 aliquots were analyzed for energy, no analytical value was accepted in which the largest value exceeded the smallest by more than 1.5% of the largest. Repeat analyses were done for 18.2 and 12.1% of the protein and energy determinations, respectively. As stated previously, no repeat analyses for moisture were done intentionally. Therefore, the standard deviations reported are truly representative of the sampling errors involved in this technique of carcass preparation. Those moisture determinations done incidentally with repeat energy determinations were significantly different ($P = 0.10$) from those done originally (Table II). That the homogenate would lose moisture upon reblending is inevitable. The importance of this very small reduction in moisture content, occurring where homogenates are reblended, upon other analytical values simultaneously determined is shown in the last 4 columns, Table II. Values for original and repeat energy determinations are shown together with those for repeat determinations corrected to reflect the reduced moisture content of the reblended homogenates. The corrected values for repeat determinations agree more nearly with values for original determinations than do the uncorrected values. Furthermore, the difference between uncorrected and corrected values attains statistical significance. Therefore, the corrected energy values for the 4 animals involved are those reported in Tables I and III. Similar corrections were not made in repeat protein analytical values because moisture determinations were not done in conjunction with their determination.

Table III shows mean carcass composition of the animals. Mean values for the treatment groups were found to be significantly different at the 5% level of probability (4, p. 251) in the following: carcass moisture, 41 and 57% protein>8% protein; carcass protein, 25, 41 and 57% protein>8% protein. No significant differences were found for carcass energy.

TABLE II. Effect of Changes in Moisture of Homogenate upon Analytical Values for Energy.

	Analytical results—wet carcass basis						
	Moisture (%)			Energy (calories/g)			
					Repeat det'n		
No. animals	Original det'n	Repeat det'n	Difference, orig. – repeat	Original det'n	Uncorr.	Corrected	Difference, uncorr. – corr.
4	61.74	60.92	+ .82 P ≅ .07*	2511.5	2544.0	2491.6	– 53.0 P ≅ .05*

* Probability that the mean difference between paired data is significant(4, p. 49).

TABLE III. Carcass Composition of Rats, Wet Tissue Basis.

Animals			Component		
No.	Description	Body wt (g)*	Moisture (%)*	Protein (%)*	Energy (calories/g)*
5	Control	84.6 ± 10.1	70.9 ± 1.3	18.73 ± .09	1689.0 ± 115.0
7	Exp: 8% protein diet	170.0 ± 9.4	60.8 ± 2.0	18.57 ±1.11	2621.5 ± 246.0
7	25% " "	239.1 ± 12.8	62.4 ± 1.5	20.93 ± .44	2431.3 ± 144.0
7	41% " "	231.3 ± 13.9	63.0 ± 1.8	20.90 ± .91	2345.3 ± 201.0
7	57% " "	207.9 ± 7.2	64.3 ± 1.8	20.82 ± .91	2232.9 ± 240.0

* Mean ± standard deviation among animals.

Discussion. In our experience, one person by this technique can usually prepare homogenates, withdraw and weigh aliquots for 6 animals, in 3 hours.

The technique described was investigated in an effort to obtain an analytical method with the advantages enumerated by Mickelsen and Anderson(1), namely, ease of preparation, possibility of duplicate analyses for any and all constituents, and possibility of preparing homogenates for animals having a considerable range in body weight. The technique described here does not have the disadvantages of the cited method, *i.e.*, the necessity of quantitatively transferring slurry from blendor to colloid mill with attendant loss of moisture, greater expenditure of time and labor in sample preparation, and requirement for expensive equipment. Our method in addition yields analytical values with similar or smaller standard deviations.

The product of a blendor is not homogeneous enough on standing to yield satisfactory sampling results, but our data show that sampling while the blendor is in operation gives satisfactory results. A special caution must be injected. The blendor must be actively mixing the entire homogenate at time of sampling. As was pointed out(1), when the slurry almost fills the blendor, resulting in poor mixing of the entire homogenate, consistent but small variations between aliquots drawn from the top and bottom of the container are evident. As the data of Table II show, regardless of the method of producing a homogenate, upon reblending it for resampling, its moisture content should be determined, and other analytical values simultaneously arrived at should be adjusted to compensate for the changed moisture content.

No ash or fat determinations were carried out; comparison of analytical results for these substances with results of Mickelsen and Anderson(1) are impossible, as also is a statement of the magnitude of total recovery of material in the entire carcass. However, it is felt that the precision and reproducibility of energy determinations should be very similar to those of fat determinations. There is every reason to assume that ash determinations should not be subject to excessive sampling errors.

Summary. A method is described for sampling small animal carcasses which enables representative aliquots to be taken of the output of a Waring blendor. Before blending, the animal carcass is autoclaved to soften the tissues. Samples are withdrawn from the blendor while the equipment is in operation

and the contents of the blendor are being actively mixed. Thirty-three rat carcasses were analyzed for moisture, protein and energy using this technique of sampling. The coefficients of variation (*i.e.*, variation among samples, within animals) found for analytical values of homogenates and derived analytical values for fresh carcasses varied from 0.25 to 1.19%. The largest coefficients of variation were found for per cent protein of homogenates (1.19%) and of fresh carcasses (0.95%). Other coefficients of variation observed were: per cent moisture in homogenates and fresh carcasses, 0.25 and 0.58%; and calories/g of homogenates and fresh carcasses, 0.32 and 0.34%, respectively.

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Tracheal Muscle Contracting Action of Metabolites Produced by Air-Borne Fungi. (28549)

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Many experiments have been performed dealing with the antibiotic action of the metabolites of air-borne fungi, which greatly affect humans in their daily lives, and with the antigenic action of their crude extracts. In studying the effect of such metabolites on the living body, the authors observed the action of the metabolites on tracheal muscles of guinea pigs, for it was assumed that some special substances contained in these fungus metabolites might be connected with bronchial asthma attacks. In this paper are presented *in vitro* experiments on the action of air-borne fungus metabolites on the tracheal muscles of guinea pigs.

Methods. 1) *Tracheal muscle preparation.* Guinea pigs weighing 300-400 g were killed, the tracheas (from the sublaryngeal part as far as the bifurcation) were removed and tracheal muscle preparations were prepared according to the method of Takagi *et al.*(1).

The preparations were suspended in Magnus-tubes filled with Locke-Ringer's solution maintained at 38°C and continuously supplied with oxygen. Contraction and relaxation of the tracheal muscle were recorded on a kymograph. Each metabolite was added into the medium at various concentrations. During the experiment the medium was maintained at pH 7.2.

2) *Collection and isolation of air-borne fungi.* Dust and air-borne fungi from a room of 4 bronchial asthma patients were cultivated in Sabouraud's medium, then each fungus was cultured in the selected medium for isolation.

Results and discussion. 1) *Isolated fungi.* As shown in Table I, 120 fungus strains were isolated. 2) *Action of the culture broth on tracheal muscle.* Culture broths of all the isolated fungi were tested for their effect on the tracheal muscle preparations. The culture broths were diluted in 10 volumes of Locke-Ringer's solution when used. The culture broths were evaluated as to whether they involved contraction, relaxation, or no effect (Table II). Since the culture broth may contain various substances, no categori-