References and Notes

1. V. C. Applegate and J. W. Moffett, "The sea lamprey," *Sci. American* 192, No. 4, 36 (1955).
2. L. F. Erkkila, B. R. Smith, A. L. McLain, "Sea lamprey control on the Great Lakes–1953 and 1954," *U.S. Fish Wildlife Serv. Spec. Sci. Rept. Fisheries Ser. No. 175* (1956).
3. V.C. Applegate *et al.*, "Toxicity of 4,346 chemicals to larval lampreys and fishes," *U.S. Fish Wildlife Serv. Spec. Sci. Rept. Fisheries Ser. No. 207* (1957).
4. We are indebted to the following organizations for preparing and supplying chemical samples and for technical advice in the development of these materials for our specific use: The Dow Chemical Company; Progressive Color and Chemical Company; Diamond Alkali Company; Niagara Chemical Division, Food Machinery and Chemical Corporation; Monsanto Chemical Company; Michigan Chemical Company; chemistry department, Bucknell University; and chemistry department, University of Michigan.
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Convenient pH Stat Reaction Vessel for Small Volumes

In a series of studies in our laboratory with the *p*H stat of Jacobsen and Léonis (*1*), it became clear that the conventional beaker type of reaction vessel had several disadvantages, the main one being an irreducible minimum solution volume of about 5 to 6 ml, which is an important limiting factor in studies with precious proteins and peptides. The diameter of the beaker and, hence, its volume could not be reduced because of the large number of accessories dipping into it—the glass and calomel electrodes, external stirrer, nitrogen inlet, and others. We have overcome this problem by using a rotating test tube as the reaction vessel with a stationary, concentric glass and calomel electrode assembly (Radiometer No. GK2021). Excellent stirring, with a complete absence of foaming in protein solution, is achieved by the shearing forces between the test tube and the stationary electrode assembly, and volumes may be reduced to 1.0 ml.

As may be seen in Fig. 1, the glass test tube is housed in a Plexiglass rotor fitted with turbine blades. Both rotation of the turbine and temperature control are achieved by a small centrifugal pump which circulates water, from a thermostatically controlled bath, through a jet impinging on the turbine blades. The electrode assembly is raised and lowered by means of a rack and pinion (fitted with a stop to safeguard the electrode bulb). Only a single polyethylene capillary for delivery of acid or base dips into the reaction chamber, thus, we have a relatively unencumbered apparatus. In our work, the use of nitrogen has not been necessary because of the very small surface area of the solution, but in more critical work a polyethylene capillary for the delivery of nitrogen could easily be attached to the electrode assembly.

In summary, the following advantages have been found in the use of this reaction vessel: (i) By using a single size of test tube, volumes of 1 to 5 ml are easily accommodated, and with a larger size of tube, volumes can go to 10 ml. (ii) Stirring is rapid, smooth, and steady in rate for long periods of time, the rate being adjustable by restricting the flow through the jet. (iii) Precise temperature control is achieved. (iv) The glass test tubes are easily changed, giving convenience during a series of titrations and constantly clean glassware without the need for dismantling the apparatus.

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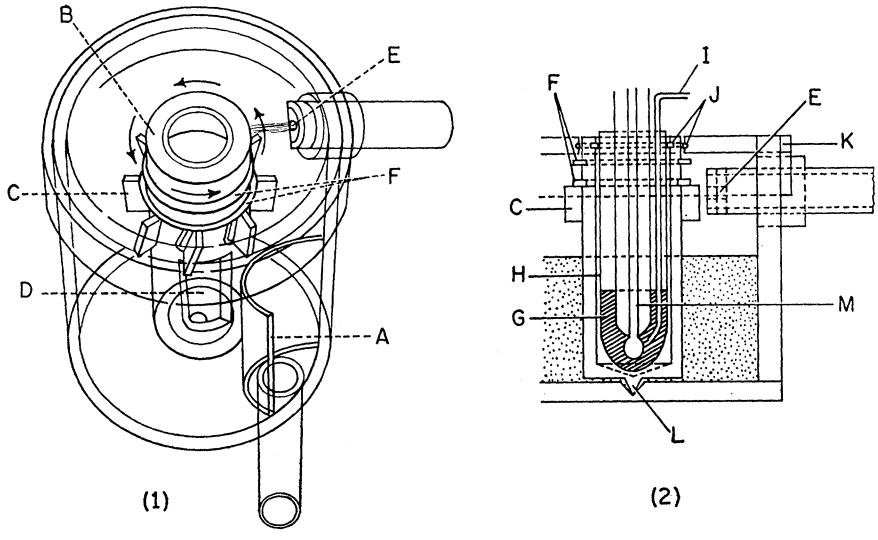

Fig. 1. Apparatus. *A*, Water outlet (designed to act both as a baffle plate and a constant-level device); *B*, rotor (Plexiglass); *C*, turbine blade; *D*, opening in rotor to facilitate rapid temperature equilibration; *E*, jet; *F*, baffle plates to prevent splashing; *G*, reaction mixture; *H*, test tube (glass); *I*, polyethylene capillary for delivery of acid and base from syringe; *J*, Teflon O-rings (the outer ring is a bearing and the inner one is an adaptor for use with various sizes of test tube); *K*, detachable lid; *L*, pivot (the pivot and its bearing should be constructed of stainless steel if extended periods of use are anticipated); *M*, radiometer electrode No. GK2021.

Reference

1. C. F. Jacobsen and J. Léonis, *Compt. rend. trav. lab. Carlsberg Ser. chim* 27, 333 (1951).

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Correlation of Potassium-40 Concentration and Fat-Free Lean Content of Hams

A rapid, objective, and nondestructive method for determining the amount of lean meat present in live animals, carcasses, and cuts of meat should prove to be of considerable value in improving the pricing efficiency involved in the marketing of livestock and meat. Objective evaluation of the amount of lean present would make it possible to set a price that would better reflect the desirability of the product from the consumer's standpoint. In addition, such a technique should help to speed the development of better grade animals for butchering, since accurate nondestructive measurements of the lean content of live animals would aid in the selection of breeding stock.

The research of Woodward *et al.* (*1*) demonstrated that the potassium content of human beings, as determined by measurement of potassium-40 (K^{40}) gamma activity by means of the Los Alamos "human counter" (*2*), was related to the body water content and, therefore, to the lean body weight of the subjects. In view of these findings, it was considered desirable to evaluate the usefulness of the K^{40} concentration as an index of the amount of lean in meat products. Since Federal pork carcass grades (*3*) are based to a considerable extent upon the degree of fatness and the proportion of lean cuts, it was decided to use hams in the study described in this report.

Fresh hams were obtained from the Animal and Poultry Husbandry Research Division of the U.S. Department of Agriculture's Agricultural Research Center at Beltsville, Maryland. One pair of hams (group A) was selected on the basis of their rather fat appearance, while the other pair (group B) appeared to contain considerably less fat. The visible portion of external fat of the hams of group A ranged from 1.2 to 1.8 in. in thickness, while the corresponding range for group B was 0.9 to 1.0 in. The hams were frozen and transported to the Los Alamos Scientific Laboratory, where the following K^{40} measurements were made

on the "human counter." The K^{40} content of group A, of group B, and of both groups together was determined. Apparently, the positioning of the hams in the counter was not of critical importance, since the total number of gamma rays per second recorded for the individual pairs of hams, after the appropriate correction had been made for background depression (21.99 and 22.29 gamma rays per second), agreed closely with the value obtained when all hams were counted simultaneously (44.3 gamma rays per second).

After the K^{40} values for the intact hams had been obtained, the skin and external fat were removed, and counting data were obtained for groups A and B. Following this, the bone and most of the seam fat were removed, and the remaining lean meat was counted for each group. The amount of crude fat present in the lean was determined (*4*) so that the K^{40} values could be related to the amount of fat-free lean present. The data obtained are presented in Table 1.

The K^{40} activity, in actually measured gamma rays per second, per pound, was obtained by dividing the value obtained for gamma rays per second by the actual weight of the sample in pounds. The K^{40} values were not adjusted by a counting efficiency factor to an absolute basis. These values varied directly with the percentage of fat-free lean present. The correlation coefficient (*5*) obtained for the correlation of these two sets of values is 0.983, indicating that the correlation is significant at the 1-percent level. The values for measured K^{40} gamma rays per second, per pound varied inversely with the percentage of fat present. A correlation coefficient of -0.966 was obtained from these values, indicating a negative correlation which is significant at the 1-percent level. The values for measured K^{40} gamma rays per second, per pound of fat-free lean were derived by dividing the value for gamma rays per second by the weight of fat-free lean in the sample. The average of these values was 1.60, with a relative standard deviation (*5*) of 4.8 percent for the total variability among different samples.

A statistical evaluation was made of the counting error involved in the counting operation (*6*). The relative standard deviation of the counting measurements in tests with pairs of hams in which the samples were counted for 20 minutes and the background was counted for 30 minutes was about 3 percent. When the four hams were counted simultaneously (58 lb of hams), counting of the sample for 13.33 minutes and of the background for 20 minutes gave a K^{40} value with a relative standard deviation for counting error of about 1.9 percent. On this basis, it may be possible to determine the K^{40} content of a 175-pound carcass (with a relative standard deviation for counting error of about 5 percent) by counting the sample for 100 seconds and the background for 200 seconds.

In order to compare the counting efficiency of the "human counter" with that of a crystal detector, the hams of group B were counted individually with an 8- by 4-inch NaI(Tl) scintillator crystal, mounted about 2 in. below the frozen ham, which rested on a piece of plywood 0.25 in. thick. Eight inches of steel shielding surrounded the sample and detector. One ham was counted for 30 minutes; this count was followed by a 30-minute count of the background. The resulting K^{40} peak for this ham was 29.4 net counts per minute, with a relative standard deviation for counting error of about 6.8 percent. The second ham of group B was counted for 60 minutes; this count was followed by a 60-minute count of the background. The resulting counting rate was 34.6 net counts per minute, with a relative standard deviation for counting error of 3.3 percent. A comparison of the counting efficiency revealed that the "human counter" detected about 21 times as many K^{40} gamma rays from hams under the conditions of the comparison made as the crystal detector did. The "human counter" has an additional advantage in that the positioning of the sample is apparently not of critical importance, while, when a crystal detector is used, the position relative to the detector is of great importance.

Table 1. K^{40} values as related to the amount of fat-free lean and fat in samples of fresh ham.

Sample	Measured K^{40} gamma rays per second, per pound	Measured K^{40} gamma rays per second, per pound of fat-free lean	Percentage of fat-free lean	Percentage of fat
		Group A		
Intact hams	0.70	1.50	47.4	40.0
Hams without skin and external fat	1.14	1.64	69.8	16.4
Lean portion	1.30	1.50	86.3	13.7
		Group B		
Intact hams	0.83	1.61	51.7	33.3
Hams without skin and external fat	1.19	1.68	70.6	14.2
Lean portion	1.48	1.68	88.5	11.5
		Groups A and B together		
Intact hams	0.76	1.56	49.4	37.1

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References

1. K. T. Woodward *et al.*, *Nature* 178, 97 (1956).
2. E. C. Anderson *et al.*, *Nucleonics* 14, 26 (1956); *IRE Trans. on Nuclear Sci. NS-3* 96 (1956).
3. *U.S. Dept. Agr. Serv. and Regulatory Announcements No. 171* (1955).
4. *Official Methods of Analysis of the Association of Official Agricultural Chemists* (Association of Official Agricultural Chemists, Washington, D.C., 1955), p. 386.
5. G. W. Snedecor, *Statistical Methods Applied to Experiments in Agriculture and Biology* (Iowa State College Press, Ames, 1946), p. 138.
6. C. L. Comar, *Radioisotopes in Biology and Agriculture* (McGraw-Hill, New York, 1955), p. 173.

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Composition of Cardiolipin

In a recent, extremely interesting communication on the composition of cardiolipin (*1*), Macfarlane and Gray have reported results which, although admittedly inconclusive, throw considerable doubt on the correctness of the presently accepted concept (*2*) that cardiolipin is a glycerylglycerophosphate derivative containing three phosphoric acid molecules linking four glycerol molecules, the latter being also esterified with one oleic and five linoleic acid residues. Indeed, Macfarlane and Gray consider that their results suggest that cardiolipin is "not a single compound, but a mixture of similar compounds varying in fatty acids." In view of this suggestion, I report, believing it to be of some interest, the results of a preliminary study in which cardiolipin was separated into a number of chromatographically distinguishable fractions each of which possessed the biological activity, as measured by the cardiolipin flocculation test (*3*), of the unfractionated cardiolipin but which differed in the fatty acid moiety.

Fifteen commercial preparations of cardiolipin (*4*) and three preparations made in this laboratory from ox-heart