optical colorimeter technique to introduce an error of ± 10 per cent. in the final result.

Creatine Determination. The conversion of creatine to creatinine was accomplished by the autoclave method of Folin.[7] Inasmuch as the creatine value is obtained by difference of the preformed and total creatinine, it was considered advisable to check the amount of creatinine destroyed and the extent of creating conversion effected by this procedure. The decomposition of creatinine was studied by submitting 1 cc samples of the creatinine zinc chloride standard to the Folin autoclave technique for varying periods of time (Table 2). A similar experiment with creatinine (C. P. Pfanstiehl) indicated an equal amount of destruction. These colorimetric findings were checked with a gravimetric procedure in which the creatinine was isolated and weighed as the flavianate in samples before and after autoclaving with 3 cc 10 per cent. flavianic acid for 20 minutes at 121° C. Three 50 mg samples in 10 cc which were not autoclaved yielded an average 191.4 mg creatinine flavianate or 50.2 mg creatinine; three autoclaved samples yielded 178.4 mg creatinine flavianate or 47.1 mg creatinine. This indicates a 6 per cent. decomposition of creatinine under the conditions of the method.

The efficacy of the creatinine conversion was tested by autoclaving 1 cc samples of a 0.1 N HCl solution containing 1 mg/cc creatine (C. P. Pfanstiehl, 32.0 per cent. N found) with 20 cc saturated picric acid solution. The extent of conversion was estimated colorimetrically (Table 2). In our experiments with the 3-hour boiling procedure of Folin[9] 83.5 to 86 per cent. creatine was found as creatinine. Due to the destruction of creatinine, it is obviously not possible to realize a complete conversion of creatine by this method.

TABLE 2

THE DECOMPOSITION OF CREATININE AND CONVERSION OF CREATINE TO CREATININE BY AUTOCLAVING AT 121° C.

Autoclaving time minutes	Creatinine decomposition Per cent.	Creatine conversion to creatinine Per cent.
10	7	67.5
20	8	86.5
40	9	92.6
60	9	93.0

Since the creatine value for adult males is normally about 10 per cent. of the creatinine output, it is understandable that if a correction is not made for the 8 to 9 per cent. creatinine decomposition incurred a low or no creatine output will be observed. It was found convenient to effect this correction by obtaining the creatine value as the difference of the total creatinine and the preformed creatinine of 1 cc samples which were simultaneously autoclaved with and without picric acid. The value so obtained is further corrected for the conversion of creatine into creatinine by the factor 1.16. The final formula for calculating creatine becomes:

$$\text{Creatine mg/day} = \frac{R_1 - R_2}{R_3} \times 1.16 \times \text{urine volume of specimen}$$

Where R_1 = total creatinine reading of 1 cc urine autoclaved with 20 cc saturated picric acid.

R_2 = preformed creatinine reading of 1 cc urine autoclaved without picric acid.

R_3 = reading of 1 cc of autoclaved standard containing 1 mg creatinine in 0.1 N HCl.

A comparison of the creatine excretion of normal adult males from 23 to 25 years of age on normal diets as determined by the Folin method and our modification showed our values to be 30 to 50 per cent. higher.

Inasmuch as creatinuria in the adult male has been consistently observed by others[6, 10, 11] who used the original Folin procedure, our creatinuria findings can not be considered specifically due to our technique, but we feel that a more accurate result is attainable by our modification.

SUMMARY

(1) It has been observed that the creatinine excretion of males and females is not an absolute value for the individual and is not influenced appreciably by the level of protein intake.

(2) Creatinuria in the adult male is a normal process and not a feminine or prepuberal male characteristic.

(3) A simple modification of the Folin method for determining creatine is described which reveals considerable quantities of creatine that may be present in urine but not detectable by the Folin technique.

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CORN OIL AND BUTTERFAT ESSENTIALLY EQUAL IN GROWTH-PROMOTING VALUE

THE Committee on Fats of the Food and Nutrition Board, National Research Council, has recently published a report on margarine.[1] In an evaluation of the relative nutritive value of different fats it comes to the conclusion that: "There is not complete agreement in the results from different laboratories as to the comparative value of different fats fed at the same level. Furthermore, with repeated experiments the results are not always the same in a given laboratory. Differences between fats will be found at one time which are statistically significant but which are not reproducible at another time with a new group of rats and a new batch of the same kind of fat."

[8] O. Folin and E. A. Doisy, *Jour. Biol. Chem.*, 28: 349, 1917.

[9] O. Folin, *Zt. f. Physiol. Chem.*, 41: 223, 1904.

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[12] O. Folin and W. Denis, *Jour. Biol. Chem.*, 11: 253, 1912.

[13] W. C. Rose, *Jour. Biol. Chem.*, 10: 265, 1911.

[14] A. B. Light and C. R. Warren, *Jour. Biol. Chem.*, 104: 121, 1934.

[1] Reprint and Circular Series, No. 118. August, 1943.

Wisconsin investigators have claimed the superiority for growth of butterfat over vegetable oils when the sole carbohydrate in the diet was lactose. They state that the younger the experimental animals, the greater are the differences obtained.[2] These conclusions were reached from a series of studies conducted with rats fed *ad libitum*.

Deuel and co-workers[3] repeated the comparison, but their results did not confirm the findings of the above mentioned investigators. They contend that the disagreement of results was due to the fact that weanling rats prefer a butter flavor and will therefore consume more of such a diet.

After weaning age, the rats received a synthetic solid milk diet whose composition was as follows:

60 parts ether extracted skim milk powder
7 " casein
6 " salt mixture
27 " butterfat or corn oil

This ration was amply supplemented with thiamin hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, choline chloride, niacinamide, ascorbic acid, calciferol, carotene and alpha-tocopherol.

An analysis of the gains in body weight, as well as of the chemical composition of the carcasses, was made

TABLE 1

SUMMARY OF PAIRED-FEEDING TESTS

	Weaned at one week*			Weaned at two weeks†		
	Butterfat	Corn oil	Diff./s.d. = "z"	Butterfat	Corn oil	Diff./s.d. = "z"
Body gains at 9 weeks, gms‡	133.7	137.5	0.63	132.8	136.8	0.45
Body lengths at 9 weeks, mm	187.2	188.9	0.50	190.1	187.9	0.94
Carcass analyses:						
Dry substance, per cent.	37.79	37.12	0.39	36.92	35.74	0.69
Ether extract, per cent.	13.79	12.26	0.59	13.03	10.02	1.65
Gross energy, cal/gm	2412.6	2339.6	0.39	2363.3	2219.4	1.00
T.N. × 6.25, per cent.	18.67	19.03	0.38	19.05	19.51	0.73

* Eight pairs, three males and five females.
† Eight pairs, four males and four females.
‡ Body gains based on empty weights.

In this study, baby rats were paired according to litter, sex and weight. One group was completely weaned at the age of one week and another at two weeks. These two groups were force- and pair-fed with the aid of a medical syringe whose needle was modified to suit the purpose. They were fed up to the age of three weeks an artificial liquid milk simulating in composition that of the rat. One member of each pair received 15 per cent. butterfat (obtained from the university creamery), while the other received 15 per cent. corn oil (Mazola).[4]

the basis of comparison between butterfat and corn oil. The average results obtained are shown in Table 1.

Statistical analysis of the data by Student's method for paired observations[5] did not show significant differences between the butterfat and corn oil groups with the exception of the ether extract and gross energy content of the carcasses in the rats weaned at the age of two weeks. This problem is being studied further.

TABLE 2

SUMMARY OF *ad libitum* FEEDING TESTS

	Experiment 1*		Experiment 2†	
	Butterfat	Corn oil	Butterfat	Corn oil
Body gains and standard error, gms‡	143.4 ± 2.72	169.8 ± 2.08	97.8 ± 2.89	91.0 ± 4.77
Food consumption and standard error, gms	361.4 ± 13.12	397.4 ± 6.71	198.0 ± 5.67	183.3 ± 7.49
Body length and standard error, mm	204.4 ± 2.01	215.2 ± 1.96	189.0 ± 1.29	182.7 ± 0.91

* Six males to each group. Three weeks experimental period.
† Five males to each group. Six weeks experimental period.
‡ Based on empty weights.

In a study involving *ad libitum* feeding with weanling rats, using the same synthetic solid milk diet, it was found that the rats fed corn oil made better gains and attained a greater body length than those fed butterfat. The food consumption, however, was likewise considerably greater. In all three instances, the differences were statistically significant. In a parallel

[2] R. K. Boutwell, R. P. Geyer, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 26: 429–437, 1943.

[3] SCIENCE, 98: 139–140, 1943.

[4] In all cases when the rats were weaned at the age of one week, bilateral cataracts occurred. These were noticed as soon as the rats opened their eyes and were verified with an ophthalmoscope. No such occurrence was found in the rats weaned at the age of two weeks. Preliminary studies indicate that a level of lactose as low as 5 per cent. in the synthetic liquid milk fed was the apparent cause.

[5] H. H. Love, "Application of Statistical Methods to Agricultural Research." Shanghai: The Commercial Press, Ltd. 1936.

study, using a ration in which 6 per cent. liver extract (1:20 paste) was included, rats fed butterfat made better, but insignificantly better (Fisher's "t" test)[6] gains over those fed corn oil. The average results of both these studies for the male rats are summarized in Table 2. The results on the females were quite similar in significance.

These studies indicate that, apart from differences in vitamin content, corn oil and butterfat are essentially equal in growth-promoting value for the rat.

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[6] F. E. Croxton and D. J. Cowden, "Applied General Statistics." New York: Prentice-Hall, Inc. 1941.

[1] J. B. Gatenby and J. D. Smyth, *Quart. Jour. Micr. Sci.*, 81, 1940.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TECHNIQUE FOR MOUNTING FREE-LIVING PROTOZOA

THE mounting of free-living protozoa on to microscope slides has always been a source of trouble to the protozoologist. The method of centrifuging after each stage in the processes of staining, dehydration, etc., suffers from several disadvantages. Firstly, the control of differentiation is difficult; for if over- or under-staining occurs, all the specimens in the tube are affected, and the whole must be re-treated. Secondly, since the organisms have to be centrifuged a considerable number of times in their passage through alcohols, stains, etc., it means that the cells are frequently distorted and true cytological pictures are not obtained. This is especially the case in dividing protozoa, where the protoplasm is less viscid than usual.[1]

Several methods of fixing protozoa to slides have been suggested,[2] but are unsatisfactory for one reason or another. The following method, recently developed by the writer, fixes the protozoa very securely to the slide and is simple and effective to use in practice.

The organisms are fixed in Schaudinn, and brought through 70 per cent. and 90 per cent. into absolute alcohol by gentle centrifuging. A small drop of albumen is placed on a clean slide, and a very thin film produced by smearing it with the edge of another slide —exactly as in the preparation of a blood film. A drop of the concentrated organisms is allowed to fall on to the film of albumen from a fine pipette held about an inch above the slide. The combined action of the dropping force and the rapid coagulation of the albumen by the alcohol, immediately causes the organisms to be fixed securely to the slide. These slides are then placed in absolute alcohol, and treated as ordinary sections.

This method avoids the difficulties mentioned above; the small amount of centrifuging necessary in the preliminary concentrating never being sufficient to damage the cells. The film of albumen, too, is so thin that it causes no interference with the staining reactions of the protozoa.

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ON QUIETING PARAMECIUM WITH METHYL CELLULOSE

MARSLAND[1] has suggested an excellent method of quieting Paramecia for study by the elementary student, using Dow "Methocel," or methyl cellulose in 10 per cent. aqueous solution.

We have found in our laboratory a slight modification of Marsland's method to be even more satisfactory for our purposes. Since 10 per cent. was a little too viscous, we tried 5 per cent., and we suggest this procedure: Make a small ring of 5 per cent. Methocel, slightly smaller than the cover glass to be used. Into the center of the ring place a small drop of medium containing Paramecia. Add a cover glass. Practice teaches one how much of each to use, but less than a full drop of each is often satisfactory with small cover glasses.

This enables the student to observe normal movement for a few minutes before diffusion of the methyl cellulose has slowed him down, and then progressively increasing viscosity gradually slows him to a completely stationary position. At this point he may be placed under an oil immersion objective, and ciliary motion studied in detail. Eventually even this slows down until the cilia appear to beat with great effort.

The Dow Company was very generous in furnishing us with the methyl cellulose.

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[2] J. B. Gatenby and T. S. Painter, "Microtomist's Vade Mecum," London, 1937.

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