aspartic acid in casein (Table I) was considerably greater than that reported by Hac and Snell(4). However, the latter was corrected only for the moisture content of the casein sample, and the true value must have been somewhat higher, if impurities other than moisture were present in significant amounts. The values obtained in the present experiments were calculated on the basis of 16% nitrogen in casein, and the nitrogen was determined in the hydrolysate. This procedure would give high values either if the true value for nitrogen in casein were significantly less than 16% or if a significant amount of the casein nitrogen were insoluble in the hydrolysate (insoluble humin was removed by filtration before the nitrogen determination was made). The use of a more painstaking procedure did not seem warranted in the present experiments because the available casein sample was not one which had been carefully prepared and highly purified.

Summary. A microbiological procedure was developed for the determination of L- and D-aspartic acids with *Leuconostoc mesenteroides* P-60. Added L- and D-aspartic acids were recovered adequately from amino acid mixtures and hydrolysates of casein and bacterial cells. Only small amounts of D-aspartic acid were detected in casein and in *Lactobacillus arabinosus* cells, but in *Streptococcus faecalis* and *Leuconostoc mesenteroides* cells about one-fourth to one-third of the total aspartic acid was of the D- configuration.

4. Hac, L. R., and Snell, E. E., *J. Biol. Chem.*, 1945, v159, 291.

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A Modified Procedure for Determining Inulin Space.* (18856)

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Although the inulin space may be a somewhat vague entity, inulin has recently come into importance as a tool in the estimation of extracellular space. The inulin space is determined by dividing the total amount of inulin present in the animal by the plasma concentration after an equilibrium has been established between the plasma and the interstitial compartments. The simplest way to compute this quantity of retained inulin is to subtract the amount excreted up to the time of equilibration from the total amount given. Such a procedure has been criticized by Kruhøffer(1) on the grounds that the subtrahend is a relatively large fraction of the minuend and a small per cent error in either of these factors is magnified considerably in calculating the difference. Gaudino and Levitt(2) claimed that following a 2-hour inulin infusion, all the inulin is excreted within 4-6 hours. Therefore, they tried to obviate the difficulty inherent in the original method by computing inulin space as the ratio of the quantity of inulin excreted within 5 hours following termination of the infusion to the concentration of inulin in the plasma.

In using their method it was found that a much longer time was required to approach complete recovery. Therefore we have attempted to improve the accuracy and expediency of the inulin space determination by introducing a slight modification of the original method.

Methods. Thirteen experiments were performed on 10 female dogs under pentobarbital anesthesia. Pure dry inulin which was fructose and pyrogen free† was accurately weighed

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1. Kruhøffer, P., *Acta physiol. scandinav.*, 1946, v11, 16.

2. Gaudino, M., and Levitt, M. F., *Am. J. Physiol.*, 1949, v157, 387.

† Pfanstiehl inulin which was checked repeatedly against recrystallized standards from different laboratories.

TABLE I. Data from 6 Inulin Experiments in Which Post-Infusion Urine Collection Was Limited to 5 Hr.

Dog	Inulin administered, mg	Inulin excreted during infusion, % of admin.	Inulin excreted in 5 hr after infusion, % of admin.	Inulin unrecovered in 5 hr	
				% of admin.	% of remain.*
1	1932	59.5	35.2	5.3	13.2
2	1757	65	28.9	6.1	17.3
2	2022	70.6	27.3	2.1	7.3
3	2296	61.8	37	1.2	3.2
3	2417	58.1	34.8	7.1	16.9
3	2010	67.4	27.9	4.7	14.4
Avg		63.7	31.9	4.4	12.1

* Amt remaining in dog at moment infusion is terminated.

and dissolved in a known volume of warm saline. After plasma and urine blanks had been obtained this warm inulin solution was administered intravenously at a constant rate with a mercury pump over a 2-hour period. This time interval was found by Gaudino and Levitt to be adequate to attain a uniform concentration of inulin throughout the extracellular space. However, this may not be sufficient time in all cases since Leonards(3) found that 6 hours were required to reach equilibrium in 2 nephrectomized dogs following a single injection of inulin. Urine was collected during this equilibration period. In order to be certain that none of the inulin in solution had precipitated out during the infusion period the concentration of inulin in the few remaining cubic centimeters was determined by chemical analysis. This checked with the actual value within the range of error of the experimental method. Precisely at the termination of the infusion plasma samples were drawn, the bladder was washed, and further urine collections were made. The amount of inulin remaining in the infusion flask and tubing at the end of 2 hours was then measured to within 0.1 to 0.2 cc. Inulin space was calculated by dividing the difference between the amount of inulin administered and the amount excreted during the infusion, by the plasma concentration, as well as by the method of Gaudino and Levitt. In the first 6 experiments urine was collected for 5 hours after the infusion was stopped, while in the last seven experiments collection was continued for from 8-11.5 hours. All inulin determinations were made by the method of Higashi and Peters(4). As a check on the method, known amounts of dry inulin were added to urine or saline and recovery studies were carried out with each experiment. The mean of the per cent difference between the known concentration of inulin and that determined chemically was 1.81 with a S.D. of ± 1.24. Correction for dead space was not made since it has been amply shown that the capacity of the ureters and pelves vary from time to time and that appearance time does not bear a linear relationship to the rate of urine flow(5,6). Besides, this correction is only of importance in calculating the inulin space and has no bearing on the inulin recovery data.

Results. Table I lists the data compiled from the first 6 experiments. On the average 63.7% of all the inulin given was excreted during the 2 hour equilibration period, while an additional 31.9% was excreted in the following 5 hours. In none of these experiments was all the inulin recovered in 5 hours. An average of 4.4% of the total amount of inulin administered remained in the dogs at the end of 5 hours.

Expressed in per cent of the amount of inulin present in the dog at the moment the infusion was interrupted, an average value of 12.1% was obtained. Since this quantity of inulin is the numerator in the calculation

3. Leonards, J., Personal communication.

4. Higashi, A., and Peters, L., *J. Lab. and Clin. Med.*, 1950, v35, 475.

5. Berger, E. Y., Dunning, M. F., Steele, J. M., Jackenthal, R., and Brodie, B. B., *Am. J. Physiol.*, 1950, v162, 318.

6. Morales, P. A., Crowder, C. H., Fishman, A. P., Maxwell, M. H., and Gomez, D. M., *Am. J. Physiol.*, 1950, v163, 454.

TABLE II. Data from 7 Inulin Experiments in Which Post-Infusion Urine Collection Was Extended Beyond 5 Hr.

Dog	Inulin admin., mg	Inulin excreted during infusion, % of admin.	Inulin excreted in 5 hr after infusion, % of admin.	Inulin unrecovered in 5 hr: % of admin.	Inulin unrecovered in 5 hr: % of remain.*	Collection time beyond 5 hr, hr	Inulin excreted after 5 hr: % of admin.	Inulin excreted after 5 hr: % of remain.*	Unrecovered inulin: % of admin.	Unrecovered inulin: % of remain.*
4	1686	73.2	22.3	4.5	17	5	2.8	10.4	1.7	6.6
5	1862	59.9	30.3	9.8	24.4	5.1	3.1	7.8	6.7	16.6
6	1707	70.7	25.7	3.6	12.2	6.5	.3	1	3.3	11.2
7	2163	73.7	21.4	4.9	18.8	6.3	1.4	5.4	3.5	13.4
8	1914	72	26.5	1.5	5.2	3.3	1.7	6	−.2	−.8
9	2290	58.8	32.4	8.8	21.5	3	4.1	9.9	4.7	11.6
10	2079	46.4	39.5	14.1	26.3	3.2	4.7	8.8	9.4	17.5
Avg		65	28.3	6.7	17.9	4.6	2.6	7	4.1	10.9

* Amt remaining in dog at moment infusion is terminated.

of the inulin space, an equivalent error is incurred in this determination.

In Table II data are recorded from a second group of experiments in which urine was collected for longer than 5 hours. In this series too, inulin recovery was incomplete after 5 hours. The average amount missing is greater than in the first 5 experiments, while the amount of inulin recovered after 5 hours was 7% of that present in the dog at the time of equilibration.

In one experiment (dog 8) inulin recovery was complete in 8.3 hours while in the other 6, significant quantities of inulin remained in the dog after 8.0 to 11.5 hours of urine collection. Inulin space determinations, using the inulin recovered in 8.0 to 11.5 hours after the infusion, show an average error of 10.9%.

These data show complete inulin recovery was not attained in any experiment in 5 hours and in only one out of 7 experiments in 8 to 11 hours, which is in keeping with the fact that inulin is excreted exponentially, approaching complete elimination asymptotically(7,8).

Discussion. There are two distinct advantages in calculating the inulin space as we have done. First of all the error is smaller. When the inulin space is computed using the amount of inulin collected in 5 hours as representing the total amount remaining at the time of equilibration, an error of 3.2 to 26.3 averaging 15% was obtained in 13 experiments. To this must be added the error of the method for inulin determination in urine. Extension of the urine collection period to 8 to 11 hours reduces the average error to 10.9%. However, by accurately weighing the dry inulin and dissolving it in a known quantity of saline, the amount administered can be determined within a 0.1% error. Using the method of Higashi and Peters(4) the average error in determining the inulin concentration in the urine excreted during the infusion was found to be 1.81%. Since two-thirds of the administered inulin is excreted during the infusion, a total error of 3.8% is obtained in the calculation of the unexcreted inulin and consequently an equal error in the estimation of the inulin space. Therefore the failure of the dog to eliminate all of the inulin in 5 hours of urine collection introduces an error about 3 times as great as that obtained by calculating this figure from the difference of the amount infused and the amount excreted during the infusion. Extension of the urine collection period only partially reduces the error in the method of Gaudino and Levitt, and necessitates a long drawn out experiment of over 11 hours.

Secondly, the method of differences which we have employed is less time consuming since the entire procedure is limited to the period

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necessary for equilibration to be reached. With increased extracellular volume the time for equilibration to be reached is longer and the time for post-infusion urine collection becomes extremely long, making the inulin space determinations impractical under such conditions.

No satisfactory explanation can be offered to account for the difference in inulin recovery in our hands and those of Gaudino and Levitt. The possibility that light anesthesia significantly altered the rate of diffusion of inulin in our experiments has not been excluded.

Summary. 1. Inulin recovery was found to be incomplete in five hours after the termination of a two-hour inulin infusion. Since the unrecovered inulin amounted to an average of 15% of the inulin present in the dog at the time of equilibration, an equivalent error was introduced into the calculation of the inulin space by the method of Gaudino and Levitt. 2. By administering a solution made from pure, accurately weighed, dry inulin, and determining the inulin content of the urine excreted during the infusion, the amount of inulin remaining in the dog can be computed with an average error of only 3.8%. In addition, the time needed for urine collection is greatly reduced.

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Role of Fat on Utilization of Lactose in Milk by Rats and Rabbits. (18857)

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The role of fat on the utilization of lactose or galactose by rats was reported by several workers(1-5). Rats cease to grow and die if lactose or galactose is the only source of carbohydrate. Growth is continued and death is averted if fat is included in the diet(1). Rats fed skim milk excrete galactose in urine and addition of fat prevents this loss in urine (2). The percentage of ingested galactose excreted in the urine is inversely proportional to the percentage of fat in the diet(4). It is, however, not clear how fat helps in the utilization of galactose. In a normal animal ingested galactose is converted into glycogen in the liver(6). It is probable that in the absence of fat in the diet liver can not convert galactose into glycogen. Glycogen content of the liver of skim milk-fed and whole milk-fed rats was, therefore, determined. As galactose is mainly present in the nervous tissue as a constituent of cerebroside it was of interest to study whether skim milk-fed rats could utilize galactose for the synthesis of cerebroside of the brain. To study if fat is necessary for the metabolism of galactose in other species of animals the effect of feeding skim milk on urinary excretion of galactose by rabbits was also studied.

Experimental. Effect of feeding skim milk and whole milk on urinary excretion of galactose and on glycogen deposition in the liver of rats. Male rats weighing between 68 and 98 g were taken. Eight rats were fed *ad libitum* mineralised(2) skim milk for 15 days. Each rat was kept in a metabolism cage and the urine was collected under toluene. For the next 2 days the rats were fed daily 80 cc of skim milk per rat. Nine rats were fed mineralised whole milk for 15 days and for the next 2 days each of the rats received daily 40 cc of whole milk isocaloric with 80 cc of skim milk. The rats also received 2 drops of a concentrate of vit. A, D and K twice a

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