2. It is shown that both the *N*-carbobenzyloxy derivatives and to a smaller but significant extent the free peptides are hydrolyzed by crystalline pepsin; the hydrolysis is more rapid at pH 4.0 than at pH 1.8. The bearing of these results on Bergmann's theory of the conditions determining peptic action is discussed.

3. The cysteine derivatives are more susceptible to peptic hydrolysis than the corresponding compounds containing cystine; this observation is considered in relation to the fact that pepsin attacks proteins more rapidly after denaturation which is itself accompanied by the appearance of —SH groups.

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Riboflavin Content of Canteen Meals

By MARGARET KERLY, *Department of Biochemistry, University College, London*

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At a time when diet is restricted and when the public is encouraged to supplement home supplies by eating at canteens, the dietary value of the meals served is important, especially in regard to the content of essential nutrients possibly in short supply. The estimations of riboflavin reported here formed part of a survey of meals served in canteens in the Leatherhead district.

METHODS

Riboflavin was estimated microbiologically on all meals and on three the values were checked by the rat growth method.

Preparation of samples. A complete meal was collected in the canteens as served, care being taken to see that the portions of food represented average servings. The whole meal was air-dried at 70–80°, then thoroughly mixed and finely ground. Samples of the dry powder were autoclaved with dilute HCl for 15 min. at a pressure of 15 lb.; the extracts adjusted to pH 6.8 with KOH, made to volume and filtered. Usually 2 g. of powder hydrolyzed with 25 ml. 0.01 N-HCl and diluted after neutralization to 100 ml. gave an extract of suitable strength. Extraction with 0.25 N-HCl (Barton-Wright & Booth, 1943) or water also gave satisfactory extraction, but the presence of starch in the water extracts complicated subsequent estimation of riboflavin (see below); the more dilute acid was preferred for extraction, as a larger volume, and better mixing with the sample, could be used without increase in the salt content of the extract. KOH was used to adjust the pH; it is recommended by Snell & Strong (1939) for neutralization of the growth medium. In several cases where fat was removed from the dry powder by extraction with petrol ether, riboflavin determinations on the defatted powder were not significantly

different from those on the unextracted powder. For the rat tests the dry food powder was fed as a supplement to the basal ration. In these experiments nine meals were bulked before drying and grinding to obtain sufficient quantities of food powder.

Microbiological method. The organism used was a strain of *Lactobacillus helveticus*, kindly given to us by Dr C. H. Gray and obtained by him originally from the National Collection of Type Cultures. Fresh inocula were prepared from the stock agar culture for each series of estimations as recommended by Barton-Wright & Booth (1943). The medium used was that described by Snell & Strong (1939). Xylose, nicotinic and pantothenic acids (Barton-Wright & Booth, 1943) or the liver extract used in the rat-growth experiments, which contained all B vitamins except riboflavin (Chattaway, Happold & Sandford, 1943), added to the medium, caused no increase in acid production. Starch added to the medium (0.4–1.0%) caused additional acid production in control tubes and at all concentrations of riboflavin, but did not alter the relationship between acid production and riboflavin content, i.e. the slope of the curve obtained by plotting acid produced against riboflavin content was not altered by the presence of starch. Similarly, alteration of the pH of the medium (pH 6.4–7.0) affected the amount of acid produced, but not the response to added riboflavin. As a routine extracts and media were adjusted to pH 6.8.

Fermentations were carried out in McCartney culture bottles having tops with a central hole and rubber liner (A. Gallenkamp and Co. Ltd., London). Inoculations were made by injecting through the rubber liner one drop of the saline suspension of the organism from a sterile syringe. Bottles were incubated for 24 hr. only because this shorter time was found to give a more nearly linear relationship between acid production and riboflavin content. Acid production was estimated by titrating directly into the bottle

with 0.1N-NaOH to a pH of 6.8. The end-point must be estimated within 0.2 pH unit, because the medium is well buffered over the range pH 6.2-8.0. With food extracts which may be deeply coloured it is essential to use a comparator (Barton-Wright & Booth, 1943).

The riboflavin content of extracts was estimated by determining growth at four levels of concentration (each in triplicate) and comparing the slope of the response curve with that of a similar curve obtained from determinations on a standard riboflavin solution. The amount of dried food extracted was chosen so that 1 ml. extract contained ap-

Fig. 1. Relationship between riboflavin concentration in medium and acid production measured by (a) titration with 0.1N-NaOH and (b) change in pH of the medium.

proximately 0.1 $\mu\text{g.}$ riboflavin. From the best straight line relating volume of NaOH required to neutralize the acid produced to volume of extract used, the extract equivalent to 1 ml. NaOH was determined. In a similar manner for each batch of extracts the riboflavin equivalent of 1 ml. of NaOH was determined for the standard riboflavin solution (0.1 $\mu\text{g./ml.}$). By equating the NaOH equivalent of extract and riboflavin solution the concentration of riboflavin in the extract was calculated. This method was adopted as it was found that food extracts gave a 'blank' value different from that of the standard riboflavin solutions, i.e. the curves when extrapolated backwards did not pass through the same zero value (0.3-0.6 ml. of NaOH) as that given by the control tubes containing no riboflavin. This acid production in the control tubes was not due to bacterial growth as similar values were obtained after sterilizing and incubating uninoculated medium. If the food extracts were photolyzed at an alkaline pH all growth-promoting activity was destroyed; such extracts after readjustment of pH, inoculation and incubation gave titration values similar to those obtained from control tubes containing no riboflavin. The curves obtained from starch-free HCl extracts of food

were similar to those obtained on medium containing starch. Curves from water extracts of food had a higher 'blank', but the same slope. As satisfactory results were obtained by comparison of the slope of acid production curves for food extracts and for standard riboflavin solutions, the reason for the initial increased response with the food extracts was not further investigated.

It has been suggested (Silber & Mushett, 1942; Davis, Mawson, Newland, Kon & Thompson, 1942) that acid production may be estimated by change in pH of the culture medium. This would be preferable to titration for coloured extracts, but as a suitable meter was not available until most of this survey was completed, the method was used for one extract (coffee) only. Satisfactory results were obtained between concentrations of 0.02 and 0.15 $\mu\text{g.}$ riboflavin (Fig. 1).

Rat growth method. The method was that of El Sadr, Macrae & Work (1940) and the regimen followed that described by Copping (1943), using the maize starch basal diet. Each food was fed at two levels, calculated to contain approximately 6 and 12 $\mu\text{g.}$ riboflavin, to groups of four rats; two similar control groups, fed 6 and 12 $\mu\text{g.}$ pure riboflavin respectively, were used for each food. Feeding was continued for 4 weeks and riboflavin content of the food powders calculated from the curves obtained by plotting growth response against the logarithm of the dose.

RESULTS

In a series of estimations of the riboflavin content of a number of food powders, results usually agreed within 10%, but larger variations were encountered in some cases. With one meal, consisting of beef, potatoes, cabbage and treacle tart, determinations were made on the undried sample and one and two weeks later, after drying and grinding (Table 1). Since there was no consistent fall in riboflavin content it was concluded that no loss was caused by drying and storing. Sampling difficulties prevented estimation of recovery of riboflavin added to a whole meal, but recovery from a portion of porridge, treated in the same way as the whole meals, was reasonably satisfactory (Table 1).

For three meals good agreement was found between the results of the microbiological and the rat-growth methods.

No obvious correlation was observed among the dishes included in the meals and the riboflavin content. Three meals which contained cheese all gave high values, but two of them also contained egg and bread pudding.

A few estimations were made on samples of Ceylon and Indian teas and on a sample of coffee. Extraction was made by pouring boiling water on to the tea or coffee, allowing to stand for 10 min. and then filtering off the extract. Standing for 3 min. before filtration did not give complete extraction, but further standing up to 45 min. did not increase the riboflavin content of the extract. These results are of the same order as those reported by Drummond & Moran (1944).

Table 1. *Riboflavin content of canteen meals*

	Riboflavin content (mg./meal)	
	(a) Microbio- logical method	(b) Rat- growth method
School canteens		
Beef, potato, salad, rice pudding	0.28	—
Lentil soup, potato, watercress, steamed pudding, custard	0.29	—
Corned beef, potato, cabbage, treacle tart	0.29	—
Mutton, potato, beans, watercress, steamed pudding, custard	0.30	—
Shepherd's pie, salad, chocolate pudding	0.32	—
Beef, potato, cabbage, steamed pudding, semolina sauce	0.32	0.34
Fish pie (cod), potato, cabbage, cake pudding, sauce	0.35	—
Toad-in-the-hole, potato, cabbage, prunes, custard	0.43	—
Beef, potato, cabbage, treacle tart: (a) Before drying	0.40	—
(b) After 1 week	0.35	—
(c) After 2 weeks	0.43	—
Steak pie, potato, cabbage, rice pudding, prunes	0.43	0.50
Beef, potato, salad, blanchmange, jam	0.55	—
Egg and cheese pie, fish pie (tinned salmon), potato, salad, bread pudding, custard	0.74	0.73
Egg and cheese pie, potato, salad, bread pudding, custard	0.92	—
Fish pie (tinned salmon), potato, salad, bread pudding, custard	0.93	—
British restaurant		
Hot pot, potato, cabbage, steamed pudding, custard	0.55	—
Cheese pudding, potato, salad, steamed syrup pudding	0.90	—
Industrial canteen		
Cold meat, potato, beetroot, jam roll, custard, bread, butter	0.40	—
Foodstuffs		
Porridge. 100 g. wet weight: (a) Before drying	0.017	—
(b) After drying	0.015	—
(c) After addition of 0.038 mg. riboflavin and drying	0.049	—
	µg./g.	
Indian tea: Sample 1	11	
2	8	
3	11	
Ceylon tea: Sample 1	9	
2	12	
3	16	
Coffee*	1	

* Growth estimated by pH change.

SUMMARY

The riboflavin content of a number of meals served at canteens has been estimated by the microbiological technique, and the values found confirmed on three meals by the rat-growth method of estimation.

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