This exponential rate of new enzyme formation is in marked, though not unexpected, contrast to the linear rate of the second stage in the recovery *in vivo* of erythrocyte cholinesterase.

The two forms of inhibited brain cholinesterase of different stabilities originally postulated by Davison (1955) are probably the freshly inhibited enzyme which can undergo spontaneous reactivation and the aged enzyme which cannot.

These results show that the mechanisms by which the activity of the brain cholinesterase is restored to normal after organophosphorus poisoning are precisely analogous to those which operate for inhibited erythrocyte cholinesterase, namely spontaneous reactivation and aging of the inhibited enzyme, and new enzyme synthesis.

SUMMARY

1. Recovery of tetraethyl pyrophosphate- or dimethyl *p*-nitrophenyl phosphate-inhibited rat-brain true cholinesterase *in vivo* occurred in two stages, a rapid partial recovery being followed by a slower, and probably exponential, return to normal activity.

2. With diisopropyl phosphorofluoridate- or isopropyl methylphosphonofluoridate-inhibited rat-brain true cholinesterase only one stage was observed in the recovery *in vivo* which occurred throughout at an exponential rate.

3. The first stage in the recovery is explained in terms of spontaneous reactivation and aging of the inhibited enzyme, and the second stage is correlated with new enzyme synthesis associated with the normal turnover of brain protein.

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The Estimation of the Available Lysine in Animal-Protein Foods

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This paper describes the development of a procedure for the estimation in foods of the lysine units whose ϵ -amino groups will undergo the Sanger reaction with fluorodinitrobenzene. There is reason to think that those lysine units in the proteins of heat-processed foods whose ϵ -amino groups are bound to other groups, and so are unable to react, are likely also to be nutritionally 'unavailable' (Eldred & Rodney, 1946; Lea & Hannan, 1950; Henry & Kon, 1950). As a consequence of this, methods of analysis for lysine that begin with acid hydrolysis have been found to over-value foods as sources of this amino acid.

Our experiments have given results suggesting that chemical estimations of 'fluorodinitrobenzene-available' lysine can give a more reliable indication of the value of animal-protein foods (Carpenter, Ellinger, Munro & Rolfe, 1957; Lea, Parr & Carpenter, 1960).

Lysine is often the factor limiting the protein

quality of mixed diets for man or animals. Cereals, which are usually the staple energy source, also supply a large proportion of the protein and this cereal protein is seriously deficient in lysine. It is therefore of practical importance that the processing of high-protein foods should be controlled so that damage to the lysine is minimized. The procedure to be described has therefore been designed to be practicable for use in a routine analytical control laboratory.

Carpenter & Ellinger (1955*a, b*) used a simple procedure which proved useful for a range of animal materials, but it was affected significantly by interference from α -dinitrophenylarginine when applied to autolysed materials, and from dinitrophenol coming from breakdown of excess of dinitrofluorobenzene, if carbohydrate were present (Conkerton & Frampton, 1959). The interference was avoided by a modification (Bruno & Carpenter, 1957) in which methoxycarbonyl chloride was

used, but this treatment led to the unexpected development of a coloured histidine derivative (Carpenter, Jones & Mason, 1959).

The description of a procedure designed to overcome these difficulties, and the results of tests for interference, recovery and reproducibility with animal-protein foods, are given below. It seems from our own experiments and those of others (Baliga, Bayliss & Lyman, 1959; J. Mauron, personal communication) that the determination of chemically 'available lysine' in foods rich in carbohydrates and the nutritional significance of the figures obtained can involve some special difficulties.

EXPERIMENTAL

1-Fluoro-2:4-dinitrobenzene (FDNB) and methoxycarbonyl chloride were obtained from British Drug Houses Ltd., Poole, Dorset. The FDNB is used as a 2.5% (v/v) solution in ethanol, which is made up freshly for each determination because of the danger of its vesicant effects if spilt on the skin. It may be solid at room temperature but it can be measured out by holding the bottle in warm water and warming the pipette before use; accuracy is not essential as the reagent is used in excess.

The diethyl ether used was the 'laboratory-chemical' grade supplied by May and Baker Ltd., Dagenham, Essex, and was stored with metallic sodium before use. The pH 8.5 buffer was 8% (w/v) NaHCO_3 -8% (w/v) Na_2CO_3 (19:1, v/v) with a final adjustment with NaOH or HCl as required.

The sample used as a reference standard of ϵ -dinitrophenyl (DNP)-lysine hydrochloride (1) was prepared by Dr M. McC. Barnes, Rowett Research Institute, Aberdeen, by the procedure of Porter & Sanger (1948); (Found: C, 38.7; H, 5.18; N, 15.3; Cl, 9.69. $\text{C}_{12}\text{H}_{16}\text{O}_6\text{N}_4\cdot\text{HCl}\cdot\text{H}_2\text{O}$ requires C, 39.3; H, 5.18; N, 15.3; Cl, 9.69%). The lysine unit in this molecule represents 39.9% of its weight. As a substandard in routine assays we used a larger sample of ϵ -DNP-lysine (2) (E. I. du Pont Co., Wilmington, Delaware, U.S.A.). By comparison of its extinction with that of the reference standard of the hydrochloride, it was estimated to contain the equivalent of 45.5% of lysine. Solutions of the two samples in *N*-HCl showed a constant extinction ratio at all wavelengths between 250 and 480 $\text{m}\mu$, with the expected absorption maxima at 265 and 365 $\text{m}\mu$ (Sanger, 1949). For use as a working standard in the analytical procedure, 150–160 μM -solutions of standard 2 in *N*-HCl were stored in the dark.

α -DNP-arginine and α -benzoylhistidine methyl ester had been prepared by Dr K. Bailey and Dr F. Sanger respectively, both of the Department of Biochemistry, Cambridge.

Test materials. A sample of threefold re-crystallized bovine insulin (Boots Pure Drug Co. Ltd., Nottingham), as used earlier by Sanger (1945), was used as a reference material.

As an example of a partially hydrolysed protein that might give rise to special interfering products, 0.4 g. of zein (British Drug Houses Ltd.) was refluxed for 4 hr. with 6 ml. of 5*N*-HCl, then neutralized and the analytical procedure was started with the addition of 0.64 g. of NaHCO_3 instead of 8 ml. of NaHCO_3 solution.

Measurement of extinction. The extinction of the final solutions was measured in an SP.1400 prism absorptiometer (Unicam Instruments Ltd., Cambridge) set at 435 $\text{m}\mu$ and with 1 cm. cells, with the instrument set to give zero extinction with water. Other workers have tested the procedure, with instruments with filters giving maximum transmission at approximately 435 $\text{m}\mu$, with closely similar results.

Analytical procedure

The principle of the procedure is first to convert lysine residues, with reactive ϵ - NH_2 groups in the food proteins, into the yellow ϵ -DNP-lysine by treatment of the material with FDNB, followed by acid hydrolysis. Ether-soluble interfering compounds are removed by extraction and the extinction of the residual aqueous layer is measured. A blank value is obtained by treatment with methoxycarbonyl chloride and extraction of the ether-soluble lysine compound which results.

Stage 1. At least 50 g. of the material to be analysed was ground so that it passed a 1/50 in. sieve. Samples were taken for the determination of N in duplicate. Two portions, each containing an estimated 30–50 mg. of N, were then taken into round-bottomed flasks and to each was added 8 ml. of 8% (w/v) NaHCO_3 . They were shaken gently to disperse the material and then left for 10 min. FDNB (0.3 ml.), previously dissolved in 12 ml. of ethanol, was added to each flask, which was then stoppered and shaken gently on a mechanical shaker for 2 hr. The stoppers were removed and the flasks stood in boiling water until there was no more effervescence, even on shaking. It may be checked that this point corresponds to a loss of weight of 10 g., i.e. the weight of ethanol added. 8.1*N*-HCl (24 ml.) was added immediately and the flasks were refluxed gently for 16 hr. with condensers adequate to prevent loss of HCl. The flasks were then disconnected after washing the condensers with water. (The condensers may still give a yellow colour on being placed in alkaline washing water, owing to the presence of dinitrophenol, a decomposition product of FDNB which is colourless in acid solution.) After the flasks had stood in ice-water for 1–2 hr. the contents were filtered through a paper such as Whatman no. 541 with water washings and the filtrate was made up to 200 ml. A portion of each filtrate was diluted again if necessary so that 2 ml. contained an estimated equivalent of 35–55 μg . of 'available lysine' from the original sample. (This usually involved a twofold to fivefold dilution.)

Stage 2. Portions (2 ml.) from each diluted filtrate were pipetted into each of two glass-stoppered tubes A and B, graduated at 10 ml., and a small conical flask C. The contents of the tubes were extracted twice with 5 ml. (approx.) portions of ether, the ether layers were discarded and the tubes were held in boiling water until effervescence from the residual ether ceased, and then they were cooled. Tube A was made up to 10 ml. with *N*-HCl and kept for the final readings.

Stage 3. The contents of flask C were titrated with 10% (w/v) NaOH, with phenolphthalein as indicator, and then discarded. The same volume of NaOH was then added to tube B, followed by 2 ml. of buffer solution, pH 8.5. Methoxycarbonyl chloride (0.045–0.055 ml.) was then added and the tube shaken vigorously to disperse and dissolve the compound. After 5–10 min. 0.75 ml. of conc. HCl was added, cautiously at first and with agitation to prevent the contents frothing over. The contents were

again extracted twice with 5 ml. of ether. (The ether washings were discarded in the standard procedure but they have been used for some of the studies reported below.) The residual ether in the aqueous layer was evaporated by standing the tube in boiling water, and the volume was made to 10 ml. with water.

Stage 4. The extinction coefficients of the contents of tubes A and B were measured in 1 cm. cells at 435 m μ (or with a filter having maximum transmission between 420 and 450 m μ if necessary). 'Reading A - reading B' was taken as the extinction due to ϵ -DNP-lysine, and was compared with the corresponding values obtained with 2 ml. of standard DNP-lysine solution passed through the procedure from stage 2 onwards, with omission only of the ether-washing in stage 2. The equivalent amount of lysine from the test food that has reacted with FDNB is calculated, with a suitable correction for losses due to hydrolysis where necessary (from the experimental results given below this appears to be multiplication by a factor of 1.09).

RESULTS

Possible interfering compounds. Starting from intact or hydrolysed proteins the only coloured, ether-insoluble DNP derivatives to be expected in the final solution at the end of stage 2 of the procedure are ϵ -DNP-lysine and α -DNP-arginine; the remaining coloured α -DNP-amino acids are ether-soluble and are removed in stage 2. The extinctions of these two compounds treated as in stage 2 (tube A) are shown in Table 1 (column A).

Treatment with methoxycarbonyl chloride, re-acidifying, extracting with ether and making each fraction to 10 ml. as in stage 3 gave the results shown in the column B. The third column X shows the extinctions obtained by evaporating the ether washings and redissolving the residues in 10 ml. of N-HCl with warming (see Bruno & Carpenter, 1957). As reported by Bailey (1957), the lysine colour is found in the ether-soluble fraction X,

whereas the arginine colour remains in B, water-soluble, so that use of the reading A - B eliminates the arginine interference.

A sample of α -benzoylhistidine methyl ester was taken through the complete procedure and in addition the ether-soluble material after methoxycarbonyl chloride treatment was taken up in N-HCl (tube X). After hydrolysis, portions were taken to give final dilutions equivalent to 30 μ M concentrations of the original material. It is seen from Table 1 that the histidine DNP derivative was colourless until coming into contact with methoxycarbonyl chloride. However, the colour was largely ether-soluble and A - B gave a value of only -0.005. This would correspond, for a protein containing 2% of histidine reacting in the same way, to an apparent 'lysine content' of -0.06%.

The colour of the histidine derivative was reduced by the final 7 min. warming period of the procedure used for tube X. More prolonged heating failed to destroy the colour completely.

Further tests with an intact zein preparation and a partially hydrolysed sample also gave apparent 'lysine' values of 0.02 and 0.10% respectively. (These compare with a range from 1.5 to 8.0% for the apparent available lysine contents of the food proteins assayed.) This suggests that interference from other amino acids, either free or combined, is small.

Recovery of added ϵ -dinitrophenyl-lysine. It is not, of course, possible to check recovery by adding pure lysine at the beginning of the procedure because this is converted with FDNB at an alkaline pH into the ether-soluble $\alpha\epsilon$ -di-DNP-lysine (Sanger, 1945). Recoveries have therefore been measured from additions of 20-30 mg. of ϵ -DNP-lysine (standard 2) after the addition of the FDNB in

Table 1. *Behaviour of zein and certain derivatives of lysine, arginine and histidine in the analytical procedure*

The estimated concentration of amino acids from zein after complete hydrolysis was based on its containing 8.8 moles of amino acid residues/kg. The X readings were obtained with the procedure described by Bruno & Carpenter (1957). With the histidine derivative the value in parentheses was obtained when the final heating period, introduced to ensure solution of the dried extract in N-HCl, was omitted.

Point of entry into procedure	Material (with final amino acid concentration in tube A)	$E_{1\text{ cm. at } 435\text{ m}\mu}$			
		Final reading in N-HCl (A)	After methoxycarbonyl chloride treatment		Difference (A - B)
			Ether-insoluble (B)	Ether-soluble (X)	
Stage 1	Blank run	0.002	0.003	0.007	-0.001
Stage 2	ϵ -DNP-lysine-HCl (1) (30 μ M)	0.203	0.002	0.201	0.201
Stage 2	α -DNP-arginine (30 μ M)	0.090	0.089	0.008	0.001
Stage 1	α -Benzoylhistidine methyl ester (30 μ M)	0.002	0.007	{ 0.103 } { (0.173) }	-0.005
Stage 1	Zein (600 μ M)	0.003	0.002	0.029	0.001
Stage 1	Partly hydrolysed zein (600 μ M)	0.018	0.014	0.017	0.004

Table 2. *Recovery of ϵ -dinitrophenyl-lysine added during analysis of foods, immediately before the acid-hydrolysis stage*

The food (0.3 g.) was analysed for lysine, both before and after the addition of 20–30 mg. of ϵ -DNP-lysine.

Food	Recovery of added DNP-lysine (%)
Fish meal (FM14)	94
Meat meal (MM5)	94
Whale-meat meal (WM7)	92
Gelatin	92
Gelatin + 10% of ribose	92
None (DNP-lysine alone)	91

triplicate runs with the foods listed in Table 2. The same foods were assayed without the addition of the standard and recoveries were estimated by measurement of the extra lysine recovered. Runs were also made with no food present. There was good agreement between the replicates in each series. The mean recovery in all the experiments was 92%. If this is used as an estimate also of the loss of ϵ -DNP-lysine formed from test foods used in the procedure, values determined should be increased by a factor of 1.09 to correct for the loss. This has been done for all the values given below.

When the procedure was applied to insulin, with this correction the value obtained was 2.49 g. of lysine/16 g. of N, which is close to the theoretical value, based on the molecular structure (Sanger & Thompson, 1953) of 2.57 g./16 g. of N. The slightly low value is to be expected here because insulin is particularly rich (in contrast with the proteins of the common foods) in histidine. As explained above, this amino acid leaves a slight residual colour in tube B.

Reproducibility of results. A statistical examination has been made of the results obtained from application of the procedure to 11 different foods. Each food had been analysed on two or more occasions, and on each occasion the analysis had been run on replicate samples to give altogether 66 individual values. A preliminary study gave a pooled standard deviation for a single value within a run of 0.10 g./16 g. of N. However, the standard deviation tended to be larger where the mean values were also large and consequently a logarithmic transformation was used for the final analysis of variance. Variance between figures obtained on different occasions (based on 17 degrees of freedom) was significantly greater than that between values obtained on the same occasion (based on 32 degrees of freedom). It was concluded that mean values (each being, as usual, from two replicates) for two foods could be considered

significantly different (at the 5% probability level) if one were at least 5% greater than the other, provided that the values were all obtained in the same run. Where the two means were obtained on different occasions the difference would have to be 8% to reach the same level of significance.

Typical values. The results obtained with 24 materials, including commercial products from slaughter-house animals, fish and whales, are set out in Table 3. The values show a wide range, from 8.6 g./16 g. of N for a sample of spray-dried blood meal down to 1.5 g./16 g. of N for a sample of feather meal. There was also a wide range in some cases between individual samples sold under the same name; for example, the two whale-meat meals gave values of 3.2 and 7.0 g./16 g. of N.

Effect of modifications of method. Table 4 sets out a comparison between the results obtained by the new procedure for ten of the same materials and the corresponding results obtained by variations of the procedure. Variation I involves following the same procedure but omitting the blank (tube B) for both unknown and standard. For eight of the ten samples the modification had the effect of increasing the values by 8–14%; for whale-meat meal, the increase was only 4% (a value checked on three occasions). For the last sample (feather meal) it was 17%, but here the absolute value was low. The ranking of the materials in order of lysine content was not altered significantly by changing to variation I.

Variation II followed the procedure (Bruno & Carpenter, 1957) for which results with pure compounds are already given under column X in Table 1, except that the final extinctions were read at 440 instead of 435 m μ . The results are also increased by the same factor (1.09) in all three variations, as an allowance for losses in the acid-hydrolysis stage. The results are again higher than with the new procedure by an average of 13%. This was to be expected because of the interference of histidine in this determination. However, there is only one change from the original ranking.

For variation III, the extracts for variation II were read at both 400 and 440 m μ , and an adjustment was made on the basis that the histidine derivative gives a ratio of extinction at these two wavelengths of 2.85:1 and the lysine derivative of 1.57:1 (Carpenter *et al.* 1959). The formula used was:

Percentage of 440 m μ colour due to lysine

$$= \frac{2.85 E_{440} - E_{400}}{(2.85 - 1.57) E_{440}} \times 100.$$

The results here were, on the average, 3% lower than those found by the new procedure but the ranking of the ten samples by this variation was unchanged.

Table 3. *Values of available lysine in animal products determined by the new procedure*

Samples coded FM, MM or WM with a number following had been distributed as part of a collaborative investigation of laboratory methods of protein-quality evaluation under the auspices of the Agricultural Research Council (Zuckerman, 1959). Samples 2 and 3 of dried pork were received from Mr D. S. Miller (Human Nutrition Research Unit, Mill Hill, London, N.W. 7), sample 5 of ossein from Dr J. E. Eastoe (British Gelatin and Glue Research Association, Holloway, London, N. 7) and sample 18 of freeze-dried cod fillets from Mr E. J. Rolfe (Ministry of Agriculture, Fisheries and Food Experimental Factory, Aberdeen). The remaining materials were commercial ones for which the origins were unknown.

Sample no.	Material analysed	Available lysine (g./16 g. of N)
1	Blood meal, spray-dried	8.6
2	Pork meat, freeze-dried	7.4
3	Pork meat, after 24 hr. at 105°	5.3
4	Gelatin	4.5
5	Ossein	3.4
6-7	Meat meals with > 13% of N (MM3; MM5)	4.1; 4.1
8-10	Meat meals with 9-10% of N (MM16; MM18; A)	2.7; 5.3; 5.2
11-12	Meat meals with < 8% of N (MM10; B)	3.9; 4.1
13	Bovine insulin	2.5
14	Hoof-and-horn meal	1.9
15	Feather meal (MM20)	1.5
16-17	Whale-meat meals (WM7; WM9)	3.2; 7.0
18	Cod fillets, freeze-dried	8.4
19	Herring meal, stored under controlled conditions	7.2
20	Herring meal, from a bulk store in which there had been spontaneous heating	5.2
21-23	Fish meals, mixed species (FM14; FM15; E)	6.6; 6.4; 6.9
24	Condensed fish solubles	4.0

Table 4. *Comparison of values obtained by the new procedure and by three variations*

Variation I consists of the standard procedure with the omission of a blank correction (tube B); II is based on the colour (read at 440 m μ) of the material extracted from the blank (Tube B) in the standard procedure; III is based on II with a correction for the interference of a histidine derivative, based on the relative extinctions of both the histidine and lysine derivatives at 400 and 440 m μ . The samples are given the numbers used in Table 3, where their descriptions can be seen.

Sample no.	Values obtained for available lysine (g./16 g. of N)			
	New procedure	I	II	III
2	7.4	8.0	8.7	7.5
19	7.2	8.0	7.8	7.3
17	7.0	7.3	8.1	6.9
21	6.6	7.5	7.3	6.5
22	6.4	7.3	6.8	6.1
3	5.3	5.7	6.3	5.2
10	5.2	5.9	5.7	5.0
12	4.1	4.7	4.4	3.9
16	3.2	3.6	3.9	3.1
15	1.5	1.7	1.6	1.4

The development of a coloured histidine derivative from the application of variation II to α -benzoylhistidine methyl ester was almost entirely prevented by treatment of the hydrolysate with sodium sulphide before the addition of methoxycarbonyl chloride (Carpenter *et al.* 1959). It has

not, however, proved possible to obtain consistent results when this pretreatment has been applied to analyses of food materials.

DISCUSSION

The usual application of the Sanger (1945) reaction of FDNB with free amino groups in proteins has been to study the molecular structure of purified materials. This is an attempt to adapt the usual procedure so that it could be applied even to crude foodstuffs and yield results indicating one aspect of their nutritive value, with only the skill and facilities to be found in a routine analytical laboratory.

Even the simple colorimetric measure of the ether-washed acid-hydrolysates of a range of animal-protein foods treated with FDNB gave results correlating well with the value of these foods as supplements to cereals in assays with chicks (Carpenter & Ellinger, 1955*a, b*). These results, and also similar results obtained later, have been encouraging for the possibilities of an analytical approach of this type.

The conditions of Sanger (1945) for the reaction with FDNB have been adopted. However, for simplicity and because of the possible presence of small peptides giving soluble DNP-peptides, the DNP-protein was not separated before the acid-hydrolysis stage. Ethanol was removed by evaporation and then the whole digest was acidified and

refluxed. Surprisingly, the presence of excess of FDNB and the non-protein components of the food does not seem to result in additional losses. Of ϵ -DNP-lysine added at the beginning of the hydrolysis stage, approximately 92% has been recovered in runs with a range of foods. This is in agreement with the findings of Sanger (1945) and Porter & Sanger (1948), who obtained recoveries of 90–95% under rather similar conditions with purified DNP-proteins freed from excess of FDNB, and concluded that ϵ -DNP-lysine was one of the most stable of the DNP-amino acids. Solomons & Irving (1958) found similar recoveries in their work with collagen, but, in contrast, Partridge & Davies (1955) have concluded that with hydrolysis of DNP-elastin there are large losses of ϵ -DNP-lysine, and that even with specially modified conditions only 65% of the added compound is recovered. Certainly there has been no such result in our own series of tests, but the results with elastin do suggest that further recovery trials would be advisable whenever a new type of food-stuff is being investigated.

Most of the coloured DNP-amino acids that might be present in our hydrolysates are removed by ether-extraction, but, in addition to ϵ -DNP-lysine, the aqueous residue may contain α -DNP-arginine, δ -DNP-ornithine, ϵ -DNP-hydroxylysine and water-soluble breakdown products from other DNP-amino acids and interaction products from the excess of FDNB and the non-protein components of the foods being tested (cf. review by Levy, 1955).

Use of the Bailey (1957) reaction with methoxy-carbonyl chloride decreases interference in the standard procedure to those molecules that change (like ϵ -DNP-lysine) to being ether-soluble with this treatment, or which remain water-soluble and change their extinction. α -DNP-arginine is unchanged. The colourless DNP-histidine derivative becomes coloured after treatment with methoxy-carbonyl chloride and a small proportion remains water-soluble, but for ordinary foods this should not decrease the true lysine value by more than 0.05 g./16 g. of nitrogen. Any reactive ornithine is measured in full, but it has not been considered a likely component of the foods considered here. Hydroxylysine would also be measured, although it cannot replace lysine as a dietary essential amino acid. In bone, tendon and skin collagens, hydroxylysine constitutes 12–21% of the total 'lysine plus hydroxylysine' present (Eastoe, 1955), and the two components react equally with FDNB through their ϵ -amino groups (Solomons & Irving, 1958). Despite this source of interference, the presence of collagen in a food will not give a misleading impression that it is of high quality as a source of lysine, because even the level of 'lysine plus hydroxylysine' is considerably lower than that of

the lysine in undiluted and undamaged-muscle protein.

The procedure does not measure lysine units with both their α - and ϵ -amino groups free, although these units are nutritionally active. With FDNB they yield di-DNP-lysine, which is ether-soluble. Where the procedure is used as a form of quality control this can actually be an advantage since most animal-protein foods that have been carefully prepared contain only very low amounts of free amino acids; the use of stale raw material in which there had been appreciable autolysis would result in lower values being recorded. Where it is required to include free lysine in the measurement, the preliminary addition of copper carbonate to block the free α -amino groups (Porter & Sanger, 1948; Solomons & Irving, 1958) may be a suitable modification, but we have not studied the effect of this step on recoveries and interference at later stages.

The application of the procedure to a range of foods has given consistently reasonable results. No figure higher than the estimated total lysine content of each food has been obtained. Low values have been associated with heat-damage. There is no evidence of proteins' remaining undenatured, and ϵ -amino groups' being unreactive for that reason, in the carefully dried products.

The two whale-meat meals listed in Table 3, WM7 and WM9, gave very different values (3.2 and 7.0 g./16 g. of nitrogen respectively). Bunyan & Price (1960) have found 8.1 g. of total lysine/16 g. of nitrogen in WM7, but the inferior quality of this sample is indicated by their further finding that its protein had a biological value for rats of only 32, as compared with a value of 69 obtained for WM9. Herring meals, from stores in which there had been heating as a result of the auto-oxidation of their unsaturated fat, also gave low values, as illustrated by sample 20 in Table 3. This has again been paralleled by a fall in nutritive value (Lea *et al.* 1960).

A comparison of results obtained by the new procedure with those obtained by three slightly different procedures (described here as variations I, II and III) that had been used earlier suggested that although they contained known sources of possible error the ranking of samples of the types tested would be little affected by the use of one procedure rather than another. Variation I has given values from 8 to 14% higher than with the new procedure for fish and meat meals in the present series. The results reported by Lea *et al.* (1960) were obtained by this procedure but had not been multiplied by 1.09 to correct for hydrolysis losses, so that we would expect them to range from 99 to 105% of the values that would be obtained by the new procedure with the correction factor applied.

Variation II, based on the previously ether-insoluble colour that becomes ether-soluble with methoxycarbonyl chloride, also gives high results. Bunyan & Price (1960) used this in its original form with no correction for destruction during hydrolysis (Bruno & Carpenter, 1957), and report values for six samples coded WM and FM that also appear in Table 3; their results are similar to ours and the samples have the same ranking in each case.

In view of the wide differences between the mean values obtained for different samples, the reproducibility of the procedure, as calculated from a statistical analysis of a large number of results, seems adequate.

SUMMARY

1. The Sanger reaction with 1-fluoro-2:4-dinitrobenzene for the determination of the free ϵ -amino groups of lysine units in purified proteins has been applied to animal-protein foods in an attempt to measure nutritional damage which may occur through the formation of enzyme-resistant linkages with ϵ -amino groups during processing.

2. The dinitrophenyl-proteins were hydrolysed with acid without separation from the digest, and the hydrolysates were ether-extracted. The measure of ϵ -dinitrophenyl-lysine was based on the further decrease in colour in the aqueous layer after digestion with methoxycarbonyl chloride and re-extraction with ether. This procedure gave a nil value for zein and the expected value for insulin.

3. Twenty-four materials, prepared in most cases as human or animal foods, gave a wide range of values from 1.5 g./16 g. of nitrogen for feather meal to 8.4 and 8.6 g./16 g. of nitrogen for freeze-dried cod fillets and spray-dried blood meal respectively. Recovery of added ϵ -dinitrophenyl-lysine was 92%.

4. Samples that had been heated and had shown decreased nutritional value in feeding tests also

showed lower 'available lysine' values in the chemical procedure.

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The Intracellular Distribution of Glycolytic and other Enzymes in Rat-Brain Homogenates and Mitochondrial Preparations

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It has been shown (Hers, Berthet, Berthet & duve, 1951) that, although most of the liver hexokinase is soluble, a proportion of the hexokinase of various mammalian tissues is associated with sub-cellular particles (Long, 1952; Crane & Sols, 1953), and the latter workers have shown that with brain

almost all of the enzyme is particulate. Glycolytic activity has been reported for various brain mitochondrial preparations (Du Buy & Hesselbach, 1956; Gallagher, Judah & Rees, 1956; Balázs & Richter, 1958; Abood, Brunngraber & Taylor, 1959). It seemed of interest therefore to determine the