renewal rate, corresponding to the steeply ascending component of the equilibrium curve; and an extremely easily hydrolysable phosphate ester with lower turnover rate. The solution of this problem however, will require further study.

SUMMARY

1. ^{32}P injected intracranially is turned over very rapidly in the rat brain. After injection for 2 min. 40 % of the newly introduced phosphate is in organic linkage. This figure increases to 70 % in 8 hr., after which it becomes approximately constant.

2. The organically bound radioactive phosphate is found, during the period of the investigation, chiefly in the form of adenosinetriphosphate and creatine phosphate. The labile phosphate groups are renewed at an approximate rate of 40 $\mu\text{g. P/min./g.}$ of brain. The value is compared with corresponding figures taken from the literature for liver and muscle.

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The Determination of N-Methyl-2-pyridone-5-carboxylic Acid in Human Urine

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Methods were recently described (Holman & de Lange, 1949) for the determination of N-methyl-2-pyridone-3-carboxylamide and N-methyl-2-pyridone-5-carboxylamide in human urine. It was shown that the former compound has no significance in nicotinic acid metabolism, but that the latter is an important metabolite. Since there is a possibility that the 2:5-amide may be converted into the corresponding acid in the body, or in urine on storage, it seemed important to devise a method for the determination of the acid and to establish definitely whether or not it is present in urine.

It was previously observed that the 2:5-acid gave the same colour reaction as was used for the determination of the 2:5-amide, but that the acid was not extracted by chloroform from aqueous sodium hydroxide and therefore could not interfere in the method for the 2:5-amide. On further experimentation it was found that the acid could be readily ex-

tracted from 10N-hydrochloric acid by one hundred volumes of ether. The amide was also extracted, but only to a small extent, the amount being negligible from normal urines. An appreciable amount was extracted from urines of exceptionally high amide content. In these cases it was possible to apply a correction for the amount of amide extracted by determining the recovery of a known amount of the amide added to the sample of urine and by determining separately the amide content of the sample. A method for the determination of the acid was worked out on these principles.

METHODS

The procedure, which was a modification of that previously described for the amide, was as follows:

Adsorption on Lloyd's reagent. Twenty-four hr. specimens of urine of 1500–2000 ml., as used for the determination of the 2:5-amide, were diluted with an equal volume of water

before analysis. Six tubes were required for each determination, two to serve as unknowns (25 ml. urine alone), two for the recovery of the acid (urine plus 0.1 mg. of the 2:5-acid), and two for the recovery of the amide (urine plus an amount of the 2:5-amide approximately equal to the amount present in 25 ml. of the urine). A standard solution of the 2:5-acid containing 0.1 mg./ml. was used (a little 25% (w/w) NH_3 was added to aid dissolution). In all other respects this stage was carried out exactly as for the determination of the 2:5-amide.

Extraction with ether. After neutralizing the eluate from Lloyd's reagent with glacial acetic acid (0.15 ml.) and evaporating to dryness in a 150 ml. glass-stoppered extraction flask, 0.25 ml. 10N-HCl and 25 ml. ether (grade for anaesthesia) were added to the residue, the mixture shaken for 5 min. and filtered through a dry paper (Whatman no. 44, 11 cm.) into another 150 ml. extraction flask. When almost all the ether had passed the filter, but before the filter became dry, a further 10 ml. ether were added to the residue in the first extraction flask, and, after shaking for several seconds, the washings were poured on to the filter. The combined filtrate and washings in the second flask were evaporated in a bath of hot water to remove the ether, and then to dryness with suction. Water (10 ml.) was added to the residue, followed by 20 ml. of a 1 : 1 mixture of CHCl_3 and ether. After shaking for 1 min. the mixture was transferred to a centrifuge tube and centrifuged; 5 ml. of the upper aqueous layer were transferred to a 7 x 1 in. Pyrex test tube. The unknown solutions and the recoveries for the acid and the amide were all treated in this way. The remaining fluid in the six centrifuge tubes was pooled, re-centrifuged and 5 ml. of the aqueous layer were transferred to another test tube to serve as a blank. The contents of all seven tubes were evaporated to dryness on a boiling water bath.

Nitration, colour development and colour measurement. These steps were carried out exactly as described in the method for the 2:5-amide, the blank being kept at room temperature while the other solutions were heated for 1 hr. at 100°.

Calculation of results. The *N*-methyl-2-pyridone-5-carboxylic acid excreted (mg./24 hr.)

$$= \frac{U - [(R_2 - U) \times v/V \times x/y]}{(R_1 - U)} \times 0.1 \times V/v,$$

where U is average optical density of unknown solutions, R_1 is average optical density of solutions containing added acid, R_2 is average optical density of solutions containing added amide, x is amide content of the specimen of urine (mg./24 hr.), determined separately by the method of Holman & de Lange (1949), y is amount (mg.) of 2:5-amide added to test the recovery of the amide, V is total volume (ml.) of 24 hr. specimen of urine after all dilutions have been made and v is volume (25 ml.) of urine taken for analysis.

RESULTS AND DISCUSSION

Accuracy of the method. Under the conditions described above, using amounts of the acid from 0.05 to 0.2 mg. and of the amide from 0.5 to 3 mg., the optical density of the final coloured solution increased in direct proportion to the amount of the acid or amide added to samples of urine. The above method of calculation of results was therefore

applicable. Approximately the same intensity of colour was given by 2 mg. of amide as by 0.1 mg. of acid.

The chief difficulty encountered in working out the method was that of obtaining a satisfactory blank. It was found that extraneous substances which gave a strong brownish colour after nitration and colour development were extracted by ether from the Lloyd's reagent eluate. Most of these substances could be removed by shaking out an aqueous solution of the ether extract with chloroform and ether, but even after this treatment the blank contained a small amount of brownish colour. No error would be incurred if the intensity of this colour were identical in the blank and test solutions, but since, in the case of some normal urines, the test solution was found to be slightly weaker in colour than the blank, a part of the colour must have been destroyed when the test solution was nitrated at 100°. Because of this destruction, apparent negative values were obtained for these urines. The error was small, however (see Table 1), and did not invalidate the broad interpretation of results.

Since samples of urine were acidified with sulphuric acid before adsorption on Lloyd's reagent, salts of the acid as well as the free acid would be determined by this method.

Application of the method. A series of typical results obtained by the present method is shown in Table 1. It is clear from the table that no detectable amount of the acid was present in urine normally, after storage for 4 weeks, or after the administration of nicotinamide.

Table 1. *Typical results obtained for the urinary excretion of N-methyl-2-pyridone-5-carboxylic acid*

Subject no.	Nature of urine sample	Excretion of <i>N</i> -methyl-2-pyridone-5-carboxylic acid (mg./24 hr.)	
		Uncorrected for amide extracted	Corrected for amide extracted
1	Normal	-1.8	-1.8
	After ingestion of 500 mg. nicotinamide	+7.0	-1.1
2	Normal	+0.3	+0.3
	After ingestion of 500 mg. nicotinamide and storage of urine for 4 weeks at room temp.	+14.4	-1.1

An experiment was made in which the urinary excretions of the 2:5-acid and amide by two healthy adult Europeans (subject 1, a male of 27 years; subject 2, a female of 36 years) were determined before and after the oral administration of 500 mg. of the acid. The results (Table 2) show that none of

the acid was converted into the amide and that 22–24 % was excreted unchanged in the urine. The fate of the remainder of the acid is as yet unknown.

Table 2. *Urinary excretion of the 2:5-acid and amide before and after oral administration of 500 mg. N-methyl-2-pyridone-5-carboxylic acid*

Subject no.	Compound determined	Urinary excretion (mg.)		Percentage of dose excreted in urine
		24 hr. specimen before dose	48 hr. specimen after dose	
1	Amide	11.0	21.1	0
	Acid	Nil	119	24
2	Amide	6.5	9.3	0
	Acid	Nil	109	22

Table 3. *Urinary excretion of the 2:5-acid and amide before and after oral administration of 500 mg. N-methyl-2-pyridone-5-carboxylamide*

Subject no.	Compound determined	Urinary excretion (mg.)		Percentage of dose excreted in urine
		24 hr. specimen before dose	48 hr. specimen after dose	
1	Amide	20.6	423	76
	Acid	Nil	Nil	0
2	Amide	9.3	404	77
	Acid	Nil	Nil	0

In a further experiment the urinary excretions of the 2:5-acid and amide by the same two subjects were determined before and after the ingestion of 500 mg. of the amide. The results (Table 3) show that none of the amide was converted into the acid and that 76–77 % of the dose was excreted unchanged in the urine in 48 hr.

These results indicate that *N*-methyl-2-pyridone-5-carboxylic acid does not play a part in human nicotinic-acid metabolism.

SUMMARY

1. A method is described for the determination of *N*-methyl-2-pyridone-5-carboxylic acid in human urine, based on preliminary purification by adsorption on Lloyd's reagent followed by extraction with ether from solution in 10*N*-hydrochloric acid, and colorimetric determination after nitration and neutralization with sodium carbonate.

2. By means of this method it is shown that *N*-methyl-2-pyridone-5-carboxylic acid is not present in urine in any detectable amount normally or after the ingestion of nicotinamide.

3. It is also shown that none of a dose of the acid is converted in the body into the corresponding amide, and that none of a dose of the amide is converted into the acid.

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Studies in the Biochemistry of Micro-organisms

81. THE COLOURING MATTERS OF *PENICILLIUM ISLANDICUM* SOPP. PART 2. CHRYSOPHANIC ACID, 4:5-DIHYDROXY-2-METHYLANTHRAQUINONE

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In a recent communication from this laboratory (Howard & Raistrick, 1949) an account was given of the isolation of the hitherto undescribed 1:4:5-trihydroxy-2-methylantraquinone from the mycelium of five out of six different strains of *Penicillium islandicum* Sopp which were examined. This colouring matter has properties which are very similar to those described for funiculosin which Igaraci (1939) isolated from laboratory cultures of *P. funiculosum* Thom. We have tried to obtain a culture of the strain of *P. funiculosum* used by Igaraci and also a specimen of funiculosin itself, but have failed to do so. We have also failed to isolate or detect any pigment having

properties similar to funiculosin or to 1:4:5-trihydroxy-2-methylantraquinone from a number of different authentic strains of *P. funiculosum*, most of which were kindly supplied by Dr Kenneth B. Raper, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. We propose, therefore, now to give the trivial name *islandicin* to the 1:4:5-trihydroxy-2-methylantraquinone from *P. islandicum*.

The sixth strain of *P. islandicum* which we examined (strain N.R.R.L. 1175), although morphologically a typical strain of *P. islandicum*, appeared to be biochemically atypical, since no *islandicin*