in vacuo. The greenish yellow crystalline residue was ground in a mortar, and was slowly added to 200 ml. conc. NH_3, with cooling. The mixture was stirred continuously for 3 hr. *N*-Methyl-2-pyridone-5-carboxylic acid amide was precipitated as a white crystalline mass, which was filtered off (yield 5 g., m.p. 201–203·5°), and purified by crystallization from absolute ethanol (pure-white needles of m.p. 201–204°). (Found: C, 54·8; H, 5·32; N, 18·2; amide N, 9·0. Calc. for $C_7H_8O_2N_2$: C, 55·2; H, 5·26; N, 18·4; amide N, 9·2%.)

(*b*) The same procedure was used as in (*a*), except that 22·5 g. of *N*-methyl-2-pyridone-5-carboxylic acid and correspondingly greater amounts of the other reagents were taken, and the time of heating with thionyl chloride was somewhat shorter (1 hr.). The yield of the crude product was 15 g. After two recrystallizations from absolute ethanol 8·5 g. of white needles of m.p. 204–206·5° were obtained.

(*c*) The procedure was the same as in (*a*), except that the time of heating with thionyl chloride was reduced to 20 min. After crystallizing once from absolute ethanol, white needles of m.p. 202–203·5° were obtained. The product, however, contained a trace of an impurity, presumably unchanged acid, which melted at 233·5°. After treatment with a solution of $NaHCO_3$ and crystallization from absolute ethanol, no trace of the high-melting impurity remained and the melting point was sharp (202–203°, uncorrected; 205·5–206·5°, corrected). After saponification for 7 hr. with 2·5N-NaOH, *N*-methyl-2-pyridone-5-carboxylic acid was recovered (m.p. 236–237°).

(*d*) *N*-Methyl-2-pyridone-5-carboxylic acid ethyl ester (5 g.) was heated for 8 hr. at 200° under a pressure of 63 kg./sq.cm. with 20 ml. methanol saturated with NH_3. After removal of methanol and NH_3 by evaporation on a steam bath, the residue was extracted once with benzene to remove unchanged ester. The amide of *N*-methyl-2-pyridone-5-carboxylic acid was isolated from the benzene-insoluble residue by crystallization from absolute ethanol. It was purified by a second crystallization from absolute ethanol; almost white crystals of m.p. 203–205° were obtained.

SUMMARY

1. Methods are described for the preparation of the amide of *N*-methyl-2-pyridone-3-carboxylic acid by the methylation and oxidation of nicotinamide, and for the preparation of *N*-methyl-2-pyridone-5-carboxylic acid by the methylation and oxidation of nicotinic acid. Methods are also given for the conversion of the former compound to the corresponding acid, and of the latter compound to the corresponding amide.

2. The properties of the two isomeric acids and amides are compared. The course of the reactions which occur in the formation of these compounds *in vitro* is discussed in relation to the biological formation of the amide of *N*-methyl-2-pyridone-5-carboxylic acid from nicotinamide in the human body.

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Absorption of 3-Methylglucose from the Small Intestine of the Rat and the Cat

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On the basis of the phosphorylation theory (Verzár & McDougall, 1936) certain sugars are absorbed from the intestine by an active process, one stage of which involves the phosphorylation of the sugars. Sugars so absorbed are thought to leave the gut more rapidly than those depending only on simple diffusion, and it is customary to judge whether or not a sugar is actively absorbed by comparing its rate of absorption with that of glucose. On this criterion, galactose and glucose are classed as being actively absorbed whilst the pentoses, such as xylose, are thought to enter by simple diffusion only. In the course of work on the synthetic 3-methylglucose, it became of interest to determine whether it was actively absorbed from the intestine. Two approaches were made: first, the rate of absorption from the

rat's small intestine was compared with that of glucose; secondly, the power of the gut to absorb the sugar against a concentration gradient was investigated in the cat. Both lines of inquiry indicated the existence of an active mechanism for the absorption of 3-methylglucose.

METHODS

Experiments on rats

In individual rats, under nembutal anaesthesia, two adjacent loops of the small intestine were isolated with ligatures; isotonic solutions of glucose and the sugar to be examined were placed in the separate loops and, after a given time, the contents were washed out and their reducing values determined by the Hagedorn & Jensen (1923) technique. From rat to rat the relative positions of the two solutions, proximal or distal to the stomach, were alternated in order to discount variations in the absorptive rate due to their position in the gut. The technique closely followed that described by Davidson & Garry (1941).

Experiments on cats

The general principle of these experiments consisted in maintaining a concentration of 3-methylglucose in the blood higher than that in the gut. If the sugar enters the blood only by simple diffusion, the absorption must take place as a result of the previous absorption of water to such an extent that the concentration of the sugar in the gut is greater than that in the blood. After a given time, then, any loss of sugar from the gut would have to be accompanied by a concentration in the gut equal to, or greater than, that in the blood, if simple diffusion were the only factor. The absorption of water could be by a specific process, or it could simply result from the colloid osmotic pressure of the plasma.

Thus an estimation of the amount of 3-methylglucose absorbed from an isolated loop, combined with a determination of the concentration of 3-methylglucose in the blood during the process of absorption, and in the gut fluid at the end of the absorptive period, should provide unequivocal evidence as to whether the sugar is actively absorbed, since a loss of sugar from the intestine, associated with a lower intestinal concentration than that in the blood, could not arise by a simple diffusion process. To reduce colloid osmotic influences to a minimum, the sugar was dissolved in the cat's own plasma. To ensure that the concentration in the blood would be the higher, the animal was given an intravenous injection of the sugar after the renal arteries had been tied. A large sample of blood was withdrawn, the plasma separated and 5 ml. inserted into an isolated loop; meanwhile, successive intravenous injections of the sugar were administered during the course of the experiment in amounts designed to maintain the blood level definitely higher than that in the gut. For control purposes a second loop was isolated and the animal's plasma, withdrawn before the injection of 3-methylglucose, inserted, and the absorption of glucose estimated.

The cats were cleared of intestinal worms with a dose of 10 mg. of arecoline hydrobromide in milk. The renal arteries were tied under nembutal anaesthesia. The femoral vein of one leg and the artery of the other were cannulated, and 10 ml. of blood withdrawn to provide the plasma for the control glucose absorption. A volume of 18 ml. of the 6% (w/v) 3-methylglucose in 6% gum acacia was injected and, after time had been allowed for equilibration, 10 ml. of blood were withdrawn and the plasma separated. The abdomen was then opened and the warm plasmas were inserted into adjoining isolated loops of the small intestine, the technique of Davidson & Garry (1940) being followed. During the whole of the procedure, the injection of 3-methylglucose was continued at the rate of about 2 ml. every 5 min. After a given interval the loops were removed and emptied, 0·1 ml. samples of the contents were taken for reducing-value estimations and the remainder, together with the washings of the gut, was made up to 25 ml. for Zeisel methoxyl determinations (Elek, 1939) of 3-methylglucose. Methoxyl and reducing values were also estimated on the blood samples.

In order to determine more accurately the changes in the sugar concentration of the gut fluid during the absorptive period, a second series of experiments was conducted in which a single loop was well filled with the plasma containing 3-methylglucose. The loop was emptied after a short absorption period, when at least 2 ml. of fluid remained unabsorbed. This permitted an accurate duplicate determination of the actual concentration of 3-methylglucose in the fluid. All estimations in this case were done by removing the glucose by yeast fermentation and estimating the non-fermentable 3-methylglucose by the Hagedorn & Jensen technique. The fermentation technique followed closely that described by Young (1938) and Somogyi (1927).

RESULTS

Experiments on rats

Sixteen rats were treated and the results calculated as the absorption in mg./g. of gut/hr. The results are shown in Table 1. If the rate of absorption of glucose be taken as 100 then that of 3-methylglucose is 84.

It was found that if the absorption took place in the proximal part of the intestine there was no difference between the caudal and cranial loops, whereas if more of the intestine was used and the more distal portion included there was a marked difference in the rates of absorption from the two loops, that from the caudal being appreciably less than that from the cranial loop. These differences, however, were eliminated in computing the results, since in eight rats the one sugar was in the caudal loop and in the other eight rats the position was reversed.

Table 1. *Absorption of sugars from rat intestine*

(Absorption rates as mg. sugar/g. of gut/hr.)

	3-Methylglucose	Glucose
Mean value	32·2	38·3
Standard error	2·06	1·60
Extreme ranges:		
Highest	44·7	47·0
Lowest	21·1	24·2

Difference = 15·9 %.

Experiments on cats

In the first series of experiments it was found that in each of three cats, in which the concentration of 3-methylglucose in the blood was kept above that in the gut, appreciable absorption did take place. The results are summarized in Tables 2 and 3. It will be seen from these tables that (*a*) the percentage absorption is comparable with that of glucose, and (*b*) the initial concentration of 3-methylglucose in the intestine was considerably less than that in the blood. The results giving the final concentration of 3-methylglucose were inconclusive in this series and probably not very accurate, since very little plasma remained in the intestine after the absorption period.

Table 2. *Percentage absorption of sugars in loops of cats' intestine*

Cat	3-Methylglucose	Glucose
I	30	40
II	38	35
V	50	60

Table 3. *Concentration of 3-methylglucose during absorption in the cat*

(Concentration in mg./100 ml. plasma.)

	Cat		
	I	II	V
Av. concentration of 3-methylglucose in blood during absorption	690	400	230
Initial concentration of 3-methylglucose in intestine	470	250	190

Table 4. *Concentration of 3-methylglucose during absorption in the cat*

Concentration of 3-methylglucose (mg./100 ml. plasma)		Cat VI	Cat VII	Cat VIII
In intestine:	Initially	219	249	231
	After absorption	189	206	178
In blood:	Before absorption	261	240	229
	After absorption	342	263	238

In the second series of experiments (Table 4) it was found that, in each of three cats in which the concentration of 3-methylglucose in the blood was maintained at or above the concentration in the intestine, an appreciable lowering of the concentration of 3-methylglucose took place.

DISCUSSION

Amongst others Cori (1925*a*, *b*) and Wilbrandt & Laszt (1933) have shown that different sugars are absorbed at different rates from the gut. In general, the hexoses are absorbed more rapidly than the pentoses. It has been suggested that the difference in the rates between these two groups of sugars is due to the fact that the hexoses (glucose and galactose) are actively absorbed, whereas the pentoses are merely absorbed by simple diffusion. Verzár & McDougall (1936) suggested that the mechanism of active absorption may be phosphorylation, a theory which is supported by the fact that glucose and galactose are phosphorylated *in vivo*, whereas the pentoses are not.

Table 5. *Absorption rates of various sugars from rat intestine*

Galactose	115	Fructose	44
Glucose	100	Pentoses	30
3-Methylglucose	84		

Table 5 shows a comparison between the rates of absorption of various sugars from the rat's intestine (Wilbrandt & Laszt, 1933) to which our results with 3-methylglucose have been added. It will be seen from this that 3-methylglucose, on the above basis, would be expected to be actively absorbed in the rat. The fact that the sugars were apparently absorbed more slowly the more distal the loop from the stomach, coupled with the evidence of Davidson & Garry (1941) that there was no difference in the rates of absorption of the pentoses in the caudal and cranial loops of the rat's intestine, suggests that the efficiency of the 'active' absorption mechanism decreases along the length of the small intestine.

In the experiments in the cat there can be little doubt that absorption did take place against a concentration gradient. It has also been shown that the concentration of sugar in the gut loop decreased during absorption so that there can be no question that absorption took place by preferential absorption of water which would upset the gradient. Bárány & Sperber (1939) have also shown that glucose can be absorbed against a concentration gradient; our results confirm this. We must, therefore, conclude that the 3-methylglucose was actively absorbed in the cat.

Previous results have shown that 3-methylglucose is not glycogenic in the rat (Campbell & Young, 1948), so that from this standpoint it would not be expected to be actively absorbed. Therefore, the

fact that it is, suggests that phosphorylation is not the mechanism of active absorption in this case. These results confirm those of Davidson & Garry (1940) in which they found that D-xylose is absorbed as rapidly as glucose from the caudal region of the small intestine of the cat. Shapiro (1947) has suggested from results with phlorrhizin and rat kidney extracts that, although phosphorylation may play a part in the absorption of sugars, it is probably far from being the only mechanism, and this is also true of the small intestines of the rat and the cat.

SUMMARY

1. In rats the rate of absorption of 3-methylglucose has been found to be about 16 % slower than that of glucose.

2. In cats 3-methylglucose has been found to be absorbed against a concentration gradient.

3. Since 3-methylglucose is not glycogenic in the rat, it is suggested that phosphorylation is not the only mechanism of active absorption either in the rat or the cat.

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Determination of Traces of Iron and Copper in Culture Media Prepared by Enzymic Digestion of Muscle Protein

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During the course of experimental work on the effect of trace metals on the toxin production of *Corynebacterium diphtheriae*, it became apparent that a very sensitive method for the determination of iron and copper was necessary. As a temporary measure, a method for iron was evolved, based on Mueller's (1938) media deferration method, consisting of adding calcium phosphate to the sample, precipitating with ammonia, and treating the precipitate with *o*-phenanthroline to develop a colour with the ferric phosphate adsorbed on the calcium phosphate. This method gave reliable and reproducible values for inorganic iron, but gives no indication of the amount of bound iron present. The problem thus resolved itself into two main steps: (*a*) incineration of the sample, and (*b*) the determination of iron and copper in the ash.

Dry ashing methods, particularly that due to Klumpp (1934), were investigated and rejected because of the significant amounts of iron and copper extracted from the ashing vessels. Silica, porcelain and pyrex crucibles all gave trouble, a fact noted by Braun & Scheffer (1940), but platinum was not tried since Bailey & McHargue (1945) stated that it gave a colour with the copper reagent similar to that given by copper itself. Wet ashing with H_2SO_4, $HClO_4$ and HNO_3 in pyrex flasks was then tried, but even after diminishing the large acid blank by redistillation of the acids, results were still erratic and high. It was not until the flasks were specially cleaned that consistent results were obtained. Wet ashing was a lengthy procedure until Smith's (1946) method was investigated, in which digestion was greatly accelerated by the use of vanadium as a catalyst. This method gave no trouble provided that the rate of heating was controlled to prevent spontaneous ignition.

Of a number of colorimetric reagents available for the determination of small quantities of iron, $\alpha\alpha'$-dipyridyl and *o*-phenanthroline appeared to be the most satisfactory from the point of view of sensitivity, stability of colour and freedom from interference, and a number of methods employing both dipyridyl (Hill, 1930; Dyer & MacFarlane, 1938; Jackson, 1938; Schulek & Floderer, 1939; Thorpe, 1941; Koenig & Johnson, 1942; Moss & Mellon, 1942; Norlin, 1943; Kitzes, Elvehjem & Schuette, 1944) and phenanthroline (Saywell & Cunningham, 1937; Fortune & Mellon, 1938; Hummel & Willard, 1938; Schaefer, 1940; Mehlig & Hulett, 1942; Borei, 1943; Bandemer & Schaible, 1944; Benne & Snyder, 1944) is described in the literature. The thiocyanate complex was rejected in view of its in-