The effect of RNA supplementation of rat diets on the composition of body fluids

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1. In a number of separate experiments, yeast RNA, mixtures of its constituent nucleosides, its constituent bases and ribose were administered orally to rats. In each instance, the resultant changes in the composition of body fluids were monitored using sensitive methods.

2. Ingestion of RNA (100 g/kg diet) caused detectable increases in intestinal ribose, inorganic phosphate, uridine, pseudouridine, uracil, inosine, uric acid and probably other purine bases. Their accumulation did not detectably affect the rate of passage of food along the digestive tract, even though some nucleosides are known to affect gut motility.

3. Although plasma levels of uric acid and uridine were higher when RNA was administered in the diet, these changes were very slight compared with those in plasma uracil, which in some experiments were increased more than 20-fold compared with control levels (300 μmol/l). Analysis of erythrocytes indicated that the internal environment of at least some cells of the body are similarly altered.

4. Analyses indicated that all dietary RNA-phosphate passed into the urine from the gut but most of the RNA-ribose was probably metabolized. Uracil and uric acid levels in the urine reflected plasma composition.

5. The effect of orally administered mixed nucleosides on blood and urine composition was similar to that of RNA, but the response to an equivalent mixture of free bases differed in several respects; cytosine, adenine and hypoxanthine appeared in urine only under these circumstances.

The background to the present study is the current interest in the possibility of producing protein food commercially from 'single-cell' sources (bacteria, yeasts, fungi, algae; see Mateles & Tannenbaum (1968) and Kihlberg (1972)). Since the rate of protein synthesis by these methods is extremely high, the product formed almost inevitably contains large quantities of RNA, chiefly of ribosomal origin (Elsworth, Miller, Whitaker, Kitching & Sayer, 1968; for review, see Brachet, 1960). If the production of 'single-cell' protein proves economical, its commercial future will mainly depend on the potentially undesirable effects of associated substances, e.g. RNA.

In previous studies we found that even high dietary levels of yeast RNA have no gross toxic effects in rats, although they cause slight disturbances in fat and carbohydrate metabolism under certain circumstances (Peers, Heaf & Davies, 1973).

Man has a peculiar difficulty in dealing with uric acid formed in the body during nucleic acid degradation (Wyngaarden, 1972) and this has resulted in the neglect of other aspects of the utilization of RNA. Therefore, only generalizations can be made about the over-all fate of dietary nucleic acid even though some of the products, particularly adenosine (for review, Burnstock, 1972), affect the activities of isolated tissue preparations (Dole, 1961; Hechter, Yoshinaga, Halkerston & Birchall, 1967; Kowal, 1970). Also, until recently, analytical techniques were both inconvenient

and of insufficient sensitivity and resolving power to monitor the numerous purine and pyrimidine derivatives released during the degradation of RNA (cf. Adams, Davis & Nakatani, 1960; Burtis & Warren, 1968; Fink & Adams, 1968; Lis, Lis & de Hackbeil, 1970; McAllan & Smith, 1973).

This paper describes the application of Simmonds' (1969) method (with modifications) to the determination of purine and pyrimidine derivatives in small samples of urine, plasma, erythrocytes and digesta. This method, together with others for the determination of nucleotides, allantoin, ribose and inorganic phosphate, was used to study changes in body-fluid composition caused by the ingestion of RNA derived from Torula yeast.

MATERIALS AND METHODS

Experimental procedures

Animals. Male Wistar rats (Charles River (UK) colony, Manston Road, Margate, Kent) were housed for at least 10 d in a thermostatically controlled room, with illumination provided by a 12 h light–dark cycle (light period 07.00–19.00 hours), and fed *ad lib.* (Rat diet 41B; Dixon and Sons Ltd, Ware, Herts.).

Administration of test compounds. The compounds to be studied were either fed to the animals as components of well-balanced diets, or were administered alone by stomach tube (under mild diethyl ether anaesthesia), as solutions, or as fine suspensions (obtained by minimal homogenization) in carboxymethyl cellulose (CMC) (20 g/l). Yeast RNA was administered as a solution in water.

The composition of the diets used is given in Table 1. As RNA represented a substantial component of the diets and the commercial preparation used contained a proportion (160 g/kg) of substances other than RNA (including sodium ions), it was added to the different diets as follows. In the two diets used in most of the experiments described, RNA was added as a supplement to the control diet at 45 and 105 g/kg (diets R_4 and R_{10} respectively).

In the third experimental diet (diet $R_{10(2)}$) RNA was added to the control diet at 115 g/kg, but was substituted specifically for cellulose, with adjustment of the casein content to minimize the effect of contaminating protein in the RNA preparation used. The RNA contents by analysis (see Table 1) were lower than the nominal amounts added.

For all experiments (Expts 1–5) rats were randomly selected and individually housed in stainless-steel metabolism cages which allowed separate collection of urine and faeces.

Expt 1. The RNA preparation used in this experiment was obtained by dissolving yeast RNA (500 mg/ml; equivalent in base composition to a mixture containing (mg/ml) 46 adenine, 53 guanine, 39 uracil, 28 cytosine) in water. The nucleoside and free base mixtures used were fine suspensions in CMC (20 g/l) (equivalent to a base composition (mg/ml) 34 adenine, 39 guanine, 29 uracil, 21 cytosine).

Four groups of six rats (160–210 g) were given the control diet *ad lib.* for 10 d. They were given 2 ml of one of the experimental preparations by stomach tube,

Table 1. *The composition (g/kg) of experimental diets fed to rats*

Diet ...	Control	Control + RNA (g/kg)		
Ingredients		40 (R_4)	100 (R_{10})	100 ($R_{10(2)}$)
Starch*	290	277	259	290
Sucrose	230	220	206	230
Casein*	150	143	134	135
Maize oil†	100	96	90	100
Cellulose‡	150	143	134	50
Minerals§	40	38	36	40
B-vitamins‖	40	38	36	40
RNA¶	—	45 (34)**	105 (78)**	115 (86)**
Chromic acid	0·1	0·1	0·1	0·1

* Purchased from British Drug Houses Ltd, Poole, Dorset.

† Obtained from Boots Pure Drugs Ltd, Nottingham. It was supplemented with a mixture of vitamins A and D (Adexolin; Glaxo Ltd, Greenford, Middlesex; supplying 15·8 mg retinol and 0·667 mg ergocalciferol/kg diet).

‡ Fibrous (CF11) obtained from Whatman Biochemicals Ltd, Maidstone, Kent.

§ Formulated according to Cuthbertson (1957). Menaphthone (Acetomenaphthone Tablets; Boots Pure Drugs Co. Ltd) was added to provide 1 mg/kg diet.

‖ Dried Brewer's Yeast, purchased from Trent Yeast Extract Co. Ltd, Burton-on-Trent, Staffordshire.

¶ Extracted from Torula yeast (Type II-s; Sigma London Chemical Co. Ltd, London) and contained (g/kg): RNA 740, DNA 24, sodium ions 30, protein 135 (Biuret method (Layne, 1957)); the remainder was accounted for by moisture. The nucleic acid fraction contained (mol/mol nucleotide) adenine 0·270, cytosine 0·209, guanine 0·279, uracil 0·240. The amino acid composition (mg/g RNA preparation; Thomas, 1970) was aspartic acid 15·9, threonine 7·0, serine 11·1, glutamic acid 25·1, glycine 11·2, alanine 9·1, cystine 2·2, valine 4·0, isoleucine 3·2, leucine 7·5, tyrosine 5·2, phenylalanine 4·3, histidine 4·4, lysine 8·8, arginine 6·2. (Tryptophan and methionine were not determined because of their instability under the conditions used for acid-hydrolysis; proline values were not quoted because the colorimeter was insensitive to its ninhydrin derivative).

** RNA content by analysis.

at the end of the 12 h light period (19.00 hours). Access to the control diet was then resumed.

Urine was collected for the next 15 h; then the animals were killed, after the removal of cardiac blood, by continued diethyl ether anaesthesia.

Expt 2. Three groups of five rats (190–225 g), which had previously been given standard laboratory diet, were fasted for 48 h and at the end of the 12 h light period (19.00 hours) were offered 10 g of the control, control + 20 g uridine/kg or $R_{10(2)}$ diets. As the amount of food provided did not satisfy their appetites, all animals consumed the entire meal within the 5 h before samples of their cardiac blood were taken.

Expt 3. Before a longer-term study of the effects of RNA on the composition of body fluids could be undertaken, a number of short-term studies were made to assess which nucleosides and bases could be determined using samples from individual animals. The experimental design was similar to that for Expt 2; individually housed animals (200–245 g, in the experiment quoted in detail) were fasted for 48 h and then given a single meal of 10 g of either the control diet or diet $R_{10(2)}$. Cardiac blood and digesta from animals in each group were sampled 5, 10 and 24 h after feeding and urine was collected during the period 0–24 h after feeding.

Expt 4. In this experiment, a longer-term study was made which involved a 19 d trial period. Three groups of twelve rats (235–295 g) were accustomed to feeding on the control diet for 5 d before being given an excess of the control diet, diet R_4, or diet R_{10} for a period of 15 h per d, starting at 19.00 hours, the end of the 12 h light period.

After the first 5 h of access to these diets, three rats from each group were killed in order to provide samples of cardiac blood and digesta. This procedure was repeated on the second, fourth and nineteenth days of the trial. As each animal was killed in order to obtain cardiac blood samples, the digestive tract was removed and the contents of its various sections retained for chromic oxide analysis. Samples of urine were obtained during the first, fourth, tenth and nineteenth 24 h periods of refeeding from rats killed on the last day of the trial. The food intake of these animals was also assessed daily throughout this period.

Expt 5. Since relatively large samples were required for the ribose analysis, a separate experiment was done to determine the utilization of this substance. Four groups of nine rats (240–300 g), which had previously been given standard laboratory diet, were fasted for 48 h and given 10 g of the control diet, control + 78 mg RNA/kg diet, control + nucleoside supplement (adenosine 20, guanosine 22, uridine 17, cytidine 15 mg/g diet), or control + ribose supplement (44 mg/g diet) immediately before the end of the 12 h light period. Three rats (all of which had consumed the entire meal) from each group were killed at 5, 11 and 16 h after they were given access to food. Digesta and blood were sampled and analysed.

Urine was collected from the surviving rats between 0 and 5 and between 0 and 11 h of the experimental period. Those urine samples from rats which received ribose were discarded because they became contaminated with faeces during acute attacks of diarrhoea which resulted from the administration of this substance.

Sampling of body fluids. Urine was collected in glass vials containing a few drops of toluene as a bacteriostat. The volume of urine obtained was measured, the urine channel of the cage was washed with water (approximately 5 ml) and the bulked sample frozen at $-20°$ until required for analysis.

When plasma alone was required, animals were anaesthetized with diethyl ether, the heart was exposed and cardiac blood was withdrawn (approximately 8 ml) into polyethylene syringes containing a small crystal of heparin; the animals were killed by continued exposure to diethyl ether. Plasma was separated from suspended cells by centrifugation and then deproteinized by ultrafiltration (800–900 *g* for 1 h) through Amicon membrane filter cones (CF10; Amicon Corp., Lexington, Mass., USA). The filtrate was then stored at $-20°$ until required for analysis.

When erythrocytes were also required, extracts were prepared as follows (using pre-cooled reagents throughout): packed cells obtained by the procedure described for plasma were quickly washed twice with 2 vol. saline (9 g sodium chloride/l) and then extracted twice with 1 vol. trichloroacetic acid solution (100 g/l). The supernatant fractions obtained by centrifugation (1500 *g* for 30 min) of the trichloroacetic acid extracts were bulked and stored at $-20°$ until required for analysis.

In experiments in which the contents of the small intestine and blood samples

were both required, cardiac blood was withdrawn as described previously and the entire length of the small intestine was then excised and transferred to a petri dish containing ice-cold saline. Within minutes, its contents were eased out by longitudinal transection and rinsing. The vigorously shaken saline suspension of intestinal contents was then centrifuged at low speed and ultrafiltered (except where specified) through Amicon membrane filter cones as described previously to yield a clear supernatant fraction which was stored at $-20°$ until required for analysis.

Similar procedures were used in experiments where, in addition to analysis of its soluble components, the passage of food along the gut was monitored. Cr_2O_3 content was determined in the solid material recovered by centrifugation. Where analysis of the gut was confined to its Cr_2O_3 content, its various sections were ashed intact.

Assessment of food consumption. Food intakes were calculated as the difference between the weights of food presented and food remaining after each 24 h period. Uneaten food paste, including that recovered from the spillage trays of the cages, was collected and dried at 105°.

Analytical procedures

Determination of purines and pyrimidines and their nucleosides in body fluids. Initially, Simmonds' (1969) method was used to determine concentrations of bases and nucleosides. However, as our analysis required, for example, better separations of uridine from inosine and of uracil from unidentified sources of u.v. absorbing materials, some modifications were made.

The modified procedure involved absorbing all the nucleic acid-related substances onto an anion-exchange column. The purines (except uric acid), pyrimidines, and their nucleosides were then eluted (free of most other u.v. absorbing substances) as a single fraction and resolved into its individual components by thin-layer chromatography using solvents described by Randerath & Randerath (1967). The spots located under u.v. light were then quantified by scanning densitometry.

Whereas urine supernatant fractions could be applied to the anion-exchange resin directly, plasma and erythrocytes required deproteinization. Ultrafiltration was found to be preferable to trichloroacetic acid precipitation for this purpose and was also used as a preliminary treatment for digesta.

Portions (usually 1·0 ml) of urine which were centrifuged immediately before use, or deproteinized samples of plasma, erythrocytes or digesta, were adjusted to pH 10 with 0·2 M-sodium bicarbonate buffer and applied to one of eight columns (300 mm × 9 mm i.d.) containing Dowex 1 × 8 anion-exchange resin (acetate form, sieved fraction from 100–200 mesh; Sigma (London) Chemical Co. Ltd, Kingston-upon-Thames, Surrey which were run in parallel. Samples were then retained on the columns when they were eluted with 10 ml 0·1 M-ammonium hydroxide, followed by 10 ml water. The first twenty 10 ml fractions eluted with 0·02 M-hydrochloric acid (1 ml/min) were collected automatically (Ultrorac Fraction Collector; LKB Instruments, Bromma, Sweden) from each column, and the extinction at 254 nm of the eluate of one column was monitored (Uvicord I; LKB Instruments). All solutions were applied to the columns using a multi-channel peristaltic pump (Technicon

Instruments Corp., Tarry Town, New York, USA) at a flow-rate of 1 ml/min. Column regeneration was automatic with two washing cycles each consisting of successive applications of 1 M-sodium hydroxide and 1 M-HCl, with 'water-washes' both between each reagent change and after the final treatment of the column with sodium acetate solution (100 g/l). The pumping of the reagents from their reservoirs was controlled by a cam-operated timer connected to four magnetic valves (Phillips Control (GB) Ltd, Farnborough, Hants), to give an over-all regeneration time of 10 h.

The eluted fractions from individual columns through which the single rather broad u.v. absorbing peak was distributed were precisely identified by spectrophotometry. These fractions, which contained the nucleosides and bases, were bulked and evaporated to small volume under reduced pressure at 40°. They were then transferred to small vials and dried in a vacuum desiccator. Each extract was dissolved in a small volume of 0·1 M-NH_4OH (usually 100 μl for digesta, plasma and erythrocytes; 1 ml for urine) for two-dimensional thin-layer chromatography using cellulose (Cmg O TLC cellulose; Reeve Angel Ltd, London) with *n*-butanol (redistilled)–water (86:14, by vol.) and *n*-butanol (redistilled)–methanol (redistilled)–water–18 M-NH_4OH (60:20:20:1, by vol.) as the developing solvents. To prepare standard curves for estimation of each derivative, mixtures of nucleosides and bases (0·1–10 μg) were chromatographed and their maximum extinction determined using a scanning densitometer fitted with a recorder (Carl Zeiss Instruments, Oberkochen, West Germany). Calibration curves, which were not linear (Fig. 1), were obtained using the areas under the 'recorder' peaks for the standard compounds.

The mean recovery of uracil standards was 85% (SD ± 7·2%) as measured by scanning densitometry. The recovery could also be monitored by adding [^{14}C]uracil to samples before application to the column (M.-L. Rabouhans and J. I. Davies, unpublished results). This procedure was particularly valuable where samples of greater than normal volume were being analysed; where there was a decrease in recoveries.

The modified procedure could be used routinely even though when large concentrations of one substance were present it limited the amount of body fluid which could be analysed by thin-layer chromatography. This resulted in a variable limit of detection.

Although scanning densitometry was not an essential procedure, it reduced the volume of body fluid required and therefore permitted the analysis of substances in individual animals.

Uracil was identified as a major constituent in urine of rats given high levels of RNA by Peers (1974) using thin-layer chromatography with a number of solvent systems and u.v. spectrophotometry of the eluted derivative in aqueous solutions of high and low pH. The latter procedure was used periodically for all derivatives.

Uric acid determination. Uric acid, which was retained on the Dowex 1 × 8 column during the preliminary purification of the nucleosides and bases, was determined directly using supernatant fractions of urine and ultrafiltered plasma and digesta samples by the method of Carr & Pressman (1962).

Allantoin. The automated procedure described by Pentz (1969) was used to determine the level of this substance in urine samples after centrifugation.

Nucleotides and polynucleotides. Polynucleotide material in digesta (which had not been subjected to ultrafiltration to remove macromolecules) was converted to mononucleotides by alkaline hydrolysis (Markham, 1957) and determined spectrophotometrically after purification by paper chromatography (Drummond & Duncan, 1970).

Ribose. The procedure involved scanning densitometry of thin-layer chromatograms of body-fluid concentrates from which a number of interfering substances had previously been removed. As this paper was being prepared, a very similar procedure was published by de Zeeuw & Dull (1975).

To remove urea from urine, 1 ml portions were made up to 5 ml with 0·1 M-sodium phosphate buffer (pH 7·0) after the addition of 5 U (one unit of activity produces 1 mg ammonia-nitrogen from urea in 5 min at pH 7·0 at 30°) urease (*EC* 3.5.1.5) (Type III; Sigma (London) Chemical Co. Ltd) and a bacteriostat (0·1 ml sodium azide solution (100 g/l)), and incubated at 37° for 2 h. Glucose was removed from 1 ml urea-free urine (adjusted to pH 5·5 with conc. HCl) by incubation overnight with 0·5 ml 0·1 M-sodium acetate buffer (pH 5·5), glucose oxidase (*EC* 1.1.3.4) (0·1 ml Fermcozyme 635 AM; Hughes and Hughes Enzymes, Romford, Essex) and 0·5 ml D-[U-^{3}H]ribose (20 nCi; Radiochemical Centre, Amersham, Bucks.).

The same procedure was used to remove glucose from 1 ml portions of plasma and digesta; sodium azide was added as above to minimize bacterial activity.

The samples were then deproteinized by ultrafiltration using Amicon membrane filter cones and deionized by passage through columns (100 mm × 6 mm i.d.) of resin (Amberlite IR 45 (OH^- form, 18–52 mesh; British Drug Houses Ltd, Poole, Dorset) – Amberlite IR 120 (H^+ form, 16–52 mesh; British Drug Houses Ltd) (1:1, w/w)). Deionized water (20 ml) was used to elute the column and the total eluate was collected, evaporated to dryness under reduced pressure and transferred to stoppered vials using three 0·5 ml portions of methanol, which was then removed by evaporation. The residue was redissolved in methanol (40–200 μl). The ^{3}H content of a 5 μl portion was determined by liquid scintillation counting, to assess the recovery of ribose (mean and SD 88 ± 8%), a 10 μl portion chromatographed with standards, using silica gel G (E. Merck AG, Darmstadt, West Germany) impregnated with phosphate (Lato, Brunelli, Ciuffini & Mazetti, 1969) and *n*-butanol (distilled)–acetone (distilled)–water (4:5:1, by vol.) as the developing solvent. The ribose was located by spraying the plates with *p*-anisidine hydrochloride, then heating in an oven at 130° for 13 min (Pridham, 1956). The extinction at 395 nm of the yellow-brown complex was determined by scanning densitometry; the recorded peak areas were used to obtain a calibration curve from the standards (this curve was not linear; Fig. 1).

The identity of the ribose was also confirmed using a series of qualitative spray reagents as described by Diamonte, Lombard, Tourn & Cassone (1971).

Inorganic phosphate. The inorganic phosphate content of 2 ml samples of urine (diluted × 100) or ultrafiltered samples of plasma (diluted × 10), small intestinal

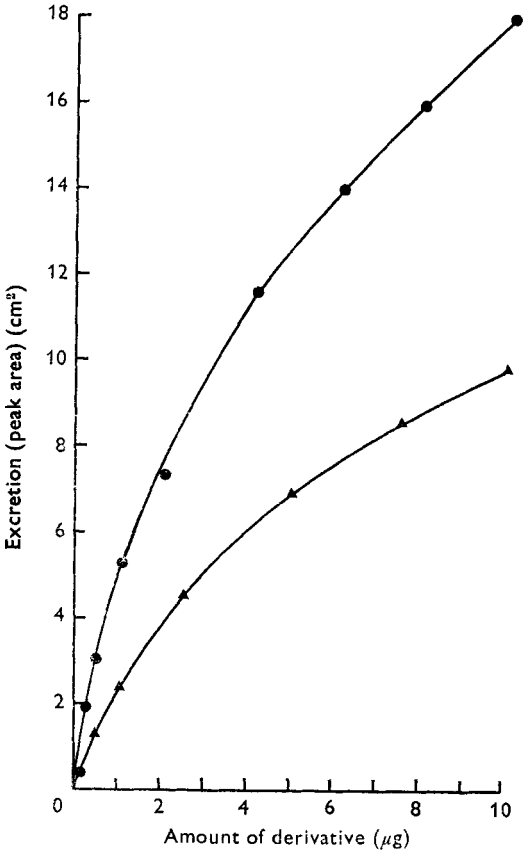

Fig. 1. Calibration curves for uracil (●—●) and ribose (▲—▲), indicating the amount of each compound (μg) applied to thin-layer chromatography plates and its corresponding extinction at 260 nm for uracil and 395 nm for ribose, as measured by scanning densitometry (area under 'recorder' peak) (cm²). For details of procedures, see pp. 385 and 387.

contents (diluted × 100) or colon contents (diluted × 100) were determined using a modification of the method of Fiske & Subbarow (1925) using an AutoAnalyzer (Technicon Instruments Corp.).

Cr_2O_3. The total solid material recovered from each section of the digestive tract was ashed and its Cr_2O_3 content determined colorimetrically by the method described by Stevenson & Clare (1963).

RESULTS

Expt 1. The effects of oral administration of a mixture of adenine, guanine, uracil and cytosine, a mixture of their ribosides, and yeast RNA on the composition of plasma and urine

The base and nucleoside composition of bulked samples of blood and urine from animals subjected to each treatment are given in Table 2.

Although the oral administration of RNA, nucleoside and base preparations all resulted in increased plasma uric acid concentrations, the marginal change was less than that found in plasma uracil concentrations, except where free base preparations

Table 2. *Expt* 1. *The effect of oral administration of RNA, mixed nucleoside and mixed purine–pyrimidine base preparations on the composition of plasma (μmol/l) after* 15 *h and urine (mg/rat) of rats during a* 15 *h collection period**

(Mean values; no. of determinations/treatment given in parentheses)

	Uric acid		Hypoxanthine		Adenine		Uracil		Cytosine		Pseudouridine	
Preparation†	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine
Control	153 (2)	1·35 (2)	< 4 (1)	< 0·1 (1)	< 4 (1)	< 0·1 (1)	11·6 (1)	0·92 (1)	< 4·5 (1)	< 0·1 (1)	< 4 (1)	0·76 (1)
Bases	242 (2)	1·96 (2)	< 4 (1)	< 0·27 (1)	< 4 (1)	< 0·35 (1)	9·8 (1)	1·28 (1)	5·0 (1)	6·9 (1)	< 4 (1)	0·89 (1)
Nucleosides	287 (2)	2·06 (2)	< 4 (1)	< 0·7 (1)	< 4 (1)	< 0·7 (1)	79 (1)	8·0 (1)	< 4·5 (1)	< 0·72 (1)	< 4 (1)	0·68 (1)
RNA	257 (2)	3·05 (2)	< 4 (1)	< 1·1 (1)	< 4 (1)	< 1·1 (1)	141 (1)	16·3 (1)	< 4·5 (1)	< 1·1 (1)	< 4 (1)	1·86 (1)

* For details of experimental procedures, see p. 382.

† Control, 20 g carboxymethyl cellulose (CMC)/l suspension; bases, a suspension in 20 g CMC/l containing (mg) adenine 68, guanine 78, uracil 58, cytosine 42; nucleosides, a suspension in 20 g CMC/l calculated to contain (mg) adenine 68, guanine 78, uracil 58, cytosine 42; RNA, 1 g in 2 ml water, calculated to contain (mg) adenine 92, guanine 106, uracil 78, cytosine 56.

Table 3. *Expt* 2. *The effect of RNA- and uridine-containing diets on nucleoside and free base concentrations (μmol/l) in erythrocytes and plasma of rats**

(Values for single determinations on bulked samples from five rats/treatment)

	Inosine		Xanthine		Hypoxanthine		Uridine		Uracil	
Diet†	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes
Control	< 0·7	1·5	< 0·7	23·0	< 0·7	88	2·9	8·2	13	18
Control + 20 g uridine/kg	< 0·7	3·0	< 0·7	22·4	< 0·7	113	7·0	9·8	317	99
Control + 86 g RNA‡/kg ($R_{10(2)}$)	< 0·7	2·2	< 0·7	15·8	< 0·7	107	4·9	12·7	101	50

* For details of experimental procedures, see p. 383.

† For details of composition, see Table 1.

‡ RNA content by analysis.

were given. The absence of increases in plasma uracil after administration of mixed base preparations was found to be a result of the interval of 15 h between administration of the dose and sampling, which is particularly inappropriate for assessing the response of plasma to free uracil (M.-L. Rabouhans and J. I. Davies, unpublished results). It is interesting, therefore, that the changes in plasma concentrations of both uracil and uric acid were so closely reflected in the urinary output of these substances. More recent work has confirmed that this, to a lesser extent, is the situation for uridine (M.-L. Rabouhans and J. I. Davies, unpublished results).

The utilization of free base preparations contrasted with that of RNA and nucleoside preparations in several other respects, particularly the appearance of cytosine in urine, and to a slight extent in plasma, of rats given free-base preparations. Furthermore, adenine and hypoxanthine accumulated in urine only in the animals treated with free bases.

Expt 2. The effects of RNA- and uridine-containing diets on the levels of nucleosides and free bases in plasma and erythrocytes

In Expt 1, the changes in plasma composition only were studied, but in Expt 2 values were obtained for plasma and erythrocytes composition (Table 3).

As would be expected from the results of Expt 1, the supplemented diets caused uracil to accumulate in plasma. Low levels of uridine were also consistently detected in plasma. Although it is not clear from these results, those of other experiments indicated that its concentration was related to the amount of its precursors in the diet (see Table 6). Uric acid concentrations were not determined.

Although the analysis of packed erythrocytes (without elimination of trapped saline medium) does not indicate precisely their internal composition, it is clear that for uridine and uracil, erythrocyte and plasma levels are at least of the same order. For uracil, this was apparent over a considerable range of concentrations. There appears to be an appreciable difference, however, between the concentration of purine derivatives in erythrocytes and plasma; inosine, hypoxanthine and xanthine being detectable only in erythrocytes.

Expt 3. The effects of the purine and pyrimidine base and the phosphate components of dietary RNA on the composition of body fluids with time after feeding

As a preliminary to longer-term studies, the effects of dietary RNA on body-fluid composition were determined at 5, 10 and 24 h after feeding in a series of experiments. Changes were found in the composition of digesta and plasma 5 h after feeding was begun. Nucleotide and polynucleotide material (approximately 8 mg/small intestine) was transiently detectable in the digesta (which had not been subjected to ultrafiltration) of animals given the control diet, and these animals also had appreciable amounts of uric acid in the gut, plasma and urine (Table 4). The increase in intestinal uric acid in response to dietary RNA was rather slight and, although similar changes were found in both plasma and urine, the determination of uric acid was somewhat affected by high concentrations of uracil which interfered with spectrophotometric measurements.

Table 4. *Expt* 3. *The effects of dietary RNA on uric acid concentrations in digesta from the small intestine, plasma and urine of rats**

(Mean values; no. of animals/treatment given in parentheses. Urine and small intestine digesta samples were analysed individually and plasma samples were bulked before duplicated analysis)

Period after feeding (h)	Diet†	Uric acid: Digesta (mg/rat)	Uric acid: Plasma (mmol/l)	Uric acid: Urine (mg/rat) per 24 h)
5	Control	2·4 (2)	0·19 (6)	nd
	Control + 86 g RNA‡/kg ($R_{10(2)}$)	3·2 (2)	0·26 (6)	nd
10	Control	2·8 (2)	0·12 (6)	nd
	$R_{10(2)}$	4·8 (2)	0·13 (6)	nd
24	Control	2·6 (2)	0·15 (6)	1·90 (8)§
	$R_{10(2)}$	2·2 (2)	0·16 (6)	4·43 (8)§
SE		±0·3		±0·20
Statistical significance of differences between treatments (analysis of variance): *P*		NS		< 0·001

NS, not significant; nd, not determined.
* For details of experimental procedures, see p. 383.
† For details of composition, see Table 1.
‡ RNA content by analysis.
§ Analysis of 24 h urine collections, obtained in a separate experiment done using the same experimental conditions.

Expt 4. *Longer-term studies of the effects of dietary RNA on the composition of body fluids*

Changes in the composition of body fluids resulting from RNA ingestion were studied on a longer-term basis, i.e. for a 19 d trial period, taking advantage of the previous finding that the responses were near their maximum at approximately 5 h after refeeding was begun.

The results of the digesta analyses (Table 5) indicate that there were appreciable levels of certain bases and nucleosides, particularly inosine, uridine and uracil in the gut of the control-fed animals. Supplementation of the diet with RNA increased the accumulation of all these substances; and the findings for guanine, xanthine and hypoxanthine were similar, although results obtained with these compounds could not be analysed statistically.

Only a weak relationship was found between the composition of digesta and that of plasma (Table 6); the only substances of this group which were detectable in the latter were uracil, uridine and uric acid. RNA ingestion caused a very marked increase in plasma uracil concentrations, which was detectable from the first day and was more than twenty times greater than the control value on day 4. It is probable that the subsequent gradual decrease in its concentration is largely attributable to a reduced rate or altered pattern of food intake (Fig. 2), although it was so marked as to suggest that some adaptation in the animal might be involved.

Although dietary RNA caused a small but highly significant increase in plasma uridine levels, no change in uric acid was detected in this experiment.

Table 5. *Expt 4. Amounts of purine and pyrimidine derivatives (mg/rat) in digesta samples from the small intestine taken at intervals from rats given RNA-containing diets for a 19 d period**

(Mean and transformed ($\log_e$) mean values for three animals/treatment)

		Uracil		Uridine		Pseudouridine					Inosine	
Day	Diet†	Mean	Transformed mean	Mean	Transformed mean	Mean	Transformed mean	Guanine Mean	Xanthine Mean	Hypoxanthine Mean	Mean	Transformed mean
1	Control	1·9	0·59	3·4	0·78	0·4‡	−1·29	< 0·07‡	0·24‡	0·21‡	1·3‡	−0·86
	R_4	1·4	0·31	2·1	0·72	0·2‡	−1·45	< 0·12‡	0·5‡	< 0·12‡	0·29‡	−1·41
	R_{10}	2·2	0·75	5·2	1·60	0·8	−0·21	< 0·22‡	2·2‡	< 0·21‡	1·7	0·29
2	Control	1·2	0·15	0·82‡	−0·89	0·2	−1·44	0·05‡	< 0·05‡	0·06‡	0·4‡	−1·42
	R_4	3·9	1·34	4·0	1·36	0·8	−0·30	0·07‡	< 0·12‡	< 0·12‡	1·1	0·04
	R_{10}	8·5	2·13	7·2	1·96	1·7	0·40	0·30	1·6	0·3‡	5·7	1·53
4	Control	1·0	0·00	1·2	0·21	0·2	−1·77	0·06‡	< 0·06‡	< 0·05‡	0·4‡	−1·36
	R_4	2·7	0·94	4·3	1·26	1·1	0·01	0·32‡	4·5‡	0·25‡	0·9	−0·10
	R_{10}	2·2	0·68	6·0	1·74	2·5	0·88	0·26	1·1‡	0·3‡	8·2	2·07
19	Control	0·6	−0·48	1·0	−0·01	0·1‡	−2·08	< 0·06‡	< 0·06‡	< 0·06‡	0·6	−0·49
	R_4	1·5	0·42	2·7	0·99	0·7	−0·50	0·06‡	0·5‡	0·2‡	1·0	−0·08
	R_{10}	2·1	0·75	7·2	1·94	2·3	0·79	0·19‡	3·4	0·5‡	9·8	2·23
SE (transformed values)			±0·09		±0·21		±0·16					±0·27
Statistical significance of differences between treatments: *P*§			< 0·001		< 0·001		< 0·001					< 0·001
Statistical significance of differences between sampling periods: *P*§			< 0·001		NS		NS					NS

NS, not significant; R_4, control + 45 (34, by analysis) g RNA/kg; R_{10}, control + 105 (78, by analysis) g RNA/kg.

* For details of experimental procedures, see p. 384.

† For details of composition, see Table 1.

‡ Groups containing values below the limit of detection; mean value therefore represents an upper limit.

§ Statistical analysis (including analysis of variance) done on transformed values to accommodate the positive relationship which exists between mean values and their variances (Snedecor & Cochran, 1967).

Table 6. *Expt* 4. *Concentrations of uracil, uridine and uric acid (μmol/l) in plasma samples taken at intervals from rats given RNA-containing diets for a* 19 *d period**

(Mean and transformed ($\log_e$) mean values; no. of animals/treatment given in parentheses)

		Uracil		Uridine		Uric acid	
Day	Diet†	Mean	Transformed mean	Mean	Transformed mean	Mean	Transformed mean
1	Control	71 (3)	3·98	6·3 (3)	1·80	134 (2)	4·88
	R_4	34 (3)	3·33	6·6 (3)	1·88	113 (2)	4·72
	R_{10}	116 (3)	4·65	9·3 (3)	2·22	125 (3)	4·80
2	Control	12 (3)	2·44	5·1 (3)	1·56	83 (3)	4·13
	R_4	70 (3)	4·25	4·9 (3)	1·55	99 (3)	4·56
	R_{10}	277 (3)	5·62	11·5 (3)	2·43	70 (3)	4·12
4	Control	13 (3)	2·54	4·4 (3)	1·46	128 (2)	4·79
	R_4	42 (3)	3·67	4·4 (3)	1·46	139 (3)	4·92
	R_{10}	305 (3)	5·43	9·0 (3)	2·17	136 (1)	4·91
19	Control	13 (3)	2·48	3·8 (3)	1·23	157 (3)	5·05
	R_4	24 (3)	3·18	6·8 (3)	1·82	151 (3)	5·02
	R_{10}	106 (3)	4·62	4·9 (3)	1·58	185 (3)	5·19
SE (transformed values)			±0·21		±0·10		±0·14
Statistical significance of differences between treatments: *P*§			<0·001		< 0·01		NS
Statistical significance of differences between sampling periods: *P*§			NS		< 0·05		< 0·01

NS, not significant; R_4, control + 45 (34, by analysis) g RNA/kg; R_{10}, control + 105 (78, by analysis) g RNA/kg.

* For details of experimental procedures, see p. 384.

† For details of composition, see Table 1.

§ Statistical analysis (including analysis of variance) done on transformed values to accommodate the positive relationship which exists between mean values and their variances (Snedecor & Cochran, 1967).

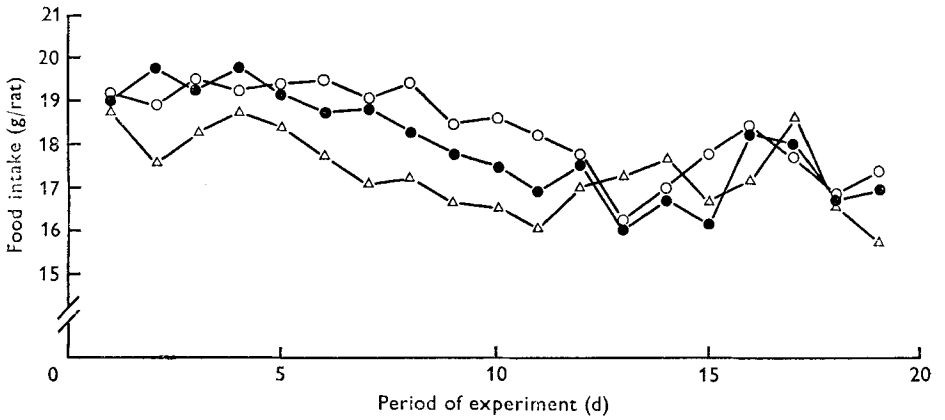

Fig. 2. Expt 4. Food intake (g/rat) of rats killed on the last day of the 19 d trial period, having been given control diet (○—○), control + 45 (34, by analysis) g RNA/kg (●—●), and control + 105 (78, by analysis) g RNA/kg (△—△). For details of experimental procedures, see p. 384; for details of diets, see Table 1.

Table 7. *Expt 4. Amounts of uracil, pseudouridine and allantoin (mg/rat) in 24 h urine samples taken at intervals from rats given RNA-containing diets for a 19 d period**

(Mean and transformed ($\log_e$) mean values for three animals/treatment)

		Uracil		Pseudouridine		Allantoin	
Day	Diet†	Mean	Transformed mean	Mean	Transformed mean	Mean	Transformed mean
1	Control	0·47	−0·76	1·6	0·47	9·7	2·27
	R_4	13·6	2·56	2·5	0·91	29·1	3·36
	R_{10}	42·4	3·69	2·9	1·02	115·0	4·59
2	Control	0·56	−0·79	1·3‡	0·21	25·2	3·15
	R_4	6·7	1·87	2·5	0·90	36·6	3·55
	R_{10}	45·0	3·80	3·5	1·13	51·8	3·95
4	Control	1·3	−0·03	1·7	0·50	44·0	3·77
	R_4	3·6	1·24	2·3	0·85	38·3	3·56
	R_{10}	37	3·43	3·7	1·29	117·7	4·68
19	Control	0·7	−0·30	1·2‡	0·07	25·0	3·21
	R_4	2·9	1·06	2·1	0·73	40·2	3·63
	R_{10}	25·0	3·20	1·7‡	0·48	61·9	4·10
SE (transformed values)			±0·11		±0·09		±0·11
Statistical significance of differences between treatments: P§			< 0·001		< 0·001		< 0·001
Statistical significance of differences between sampling periods: P§			NS		< 0·05		< 0·05

NS, not significant; R_4, control + 45 (34, by analysis) g RNA/kg; R_{10}, control + 105 (78, by analysis) g RNA/kg.

* For details of experimental procedures, see p. 384.

† For details of composition, see Table 1.

‡ Groups containing values below the limit of detection; mean value therefore represents an upper limit.

§ Statistical analysis (including analysis of variance) done on transformed values to accommodate the positive relationship which exists between mean values and their variances (Snedecor & Cochran, 1967).

The 24 h urine output of uracil was quite closely related to the composition of the blood (Table 7). Thus, the uracil was increased some 100-fold when the rats were given diet R_{10}. Furthermore, as would be anticipated from the plasma analyses, the increment in uracil output increased steeply as the amount of its precursors in the diet was increased, suggesting the existence of a threshold phenomenon.

Pseudouridine levels were slightly increased in the urine of animals given RNA-containing diets, as they were in the small intestine. This may be due to its presence as a constituent of the yeast-RNA preparation. Alternatively, since it is a component of both transfer RNA and the ribosomal RNA of higher organisms (Attardi & Amaldi, 1970), it may indicate some change in the turnover of this substance within the animal, particularly in its intestinal epithelium.

The urinary level of the main purine degradation product allantoin was consistently increased by dietary RNA.

Inorganic phosphate contents of digesta from the small intestine and colon, and

Table 8. *Expt 4. Amounts of inorganic phosphate in digesta samples from the intestines, and plasma and 24 h urine samples taken at intervals from rats given RNA-containing diets for a 19 d period**

(Mean and transformed ($\log_e$) mean values with their standard errors; no. of animals/treatment given in parentheses)

		Digesta							
		Small intestine (mg/rat)		Colon (mg/rat)		Plasma (mmol/l)		Urine‡ (mg/rat per 24 h)	
Day	Diet†	Mean	Transformed mean	Mean	Transformed mean	Mean	Transformed mean	Mean	Transformed mean
1	Control	26 (3)	3·27	3·6 (3)	1·24	4·78 (1)	1·56	61 (3)	4·11
	R_4	21 (3)	3·05	3·6 (3)	1·23	3·26 (3)	1·17	154 (3)	5·03
	R_{10}	25 (3)	3·23	3·8 (3)	1·32	3·40 (2)	1·22	345 (3)	5·78
2	Control	21 (3)	3·02	1·5 (3)	0·39	2·69 (3)	0·99	nd	nd
	R_4	30 (3)	3·41	3·2 (3)	1·16	3·02 (3)	1·10	nd	nd
	R_{10}	39 (3)	3·67	5·2 (3)	1·65	3·28 (3)	1·18	nd	nd
4	Control	20 (3)	3·01	1·6 (3)	0·46	2·90 (3)	1·05	53 (3)	3·97
	R_4	28 (3)	3·33	2·4 (3)	0·85	2·90 (3)	1·06	134 (3)	4·89
	R_{10}	30 (3)	3·41	5·2 (3)	1·65	2·84 (3)	1·04	243 (3)	5·49
10	Control	nd	nd	nd	nd	nd	nd	56 (3)	4·02
	R_3	nd	nd	nd	nd	nd	nd	144 (3)	4·96
	R_{10}	nd	nd	nd	nd	nd	nd	235 (3)	5·44
19	Control	18 (3)	2·87	1·3 (3)	0·26	2·43 (3)	0·89	52 (3)	3·94
	R_4	22 (3)	3·07	1·9 (3)	0·64	2·51 (3)	0·91	134 (3)	4·89
	R_{10}	28 (3)	3·33	5·1 (3)	1·63	2·91 (3)	1·06	268 (3)	5·57
SE (transformed values)			±0·04		±0·06		±0·04		±0·06
Statistical significance of differences between treatments: *P*§			< 0·001		< 0·001		NS		< 0·001
Statistical significance of differences between sampling periods: *P*§			< 0·001		< 0·01		< 0·001		NS

NS, not significant; nd, not determined; R_4, control + 45 (34 by analysis) g RNA/kg; R_{10}, control + 105 (78, by analysis) 5 RNA/kg.

* For details of experimental procedures, see p. 384.

† For details of composition, see Table 1.

§ Statistical analysis (including analysis of variance) done on transformed values to accommodate the positive relationship which exists between mean values and their variances (Snedecor & Cochran, 1967).

‡ 24 h urine collections were from different animals from those used for other analyses.

Table 9. *Expt 5. Levels of* D-*ribose in digesta from the small intestine, and plasma and urine of rats given diets containing RNA, mixed nucleosides or* D-*ribose**

(Mean and transformed ($\log_e$) mean values; no. of animals/treatment given in parentheses)

Sampling time (h)	Diet†	Digesta (mg/rat) Mean	Digesta (mg/rat) Transformed mean	Plasma (mmol/l) Mean	Plasma (mmol/l) Transformed mean	Urine¶ (mg/rat) Mean	Urine¶ (mg/rat) Transformed mean
5	Control	1·1 (3)	0·08	17 (3)	2·8	0·56 (3)	−0·59
	Control + 44 g D-ribose/kg diet	20 (3)	2·98	16 (3)	2·6	nd	
	Control + nucleosides‡	4·6 (3)	1·52	9 (2)	2·2	0·90 (2)	−0·11
	Control + 78 g RNA§/kg diet (R_{10})	9·4 (3)	2·19	5 (2)	1·5	0·88 (3)	−0·16
11	Control	1·7 (3)	0·54	16 (3)	2·8	1·30 (3)	0·25
	Control + 44 g D-ribose/kg diet	4·5 (3)	1·43	15 (3)	2·7	nd	
	Control + nucleosides‡	1·3 (3)	0·22	10 (3)	2·1	0·87 (3)	−0·12
	R_{10}	13·6 (3)	2·49	13 (3)	2·4	2·28 (2)	0·82
16	Control	0·55 (3)‖	−0·69	12 (3)	2·4	nd	
	Control + 44 g D-ribose/kg diet	0·67 (3)‖	−0·56	20 (3)	2·8	nd	
	Control + nucleosides‡	0·86 (3)‖	−0·40	18 (3)	2·8	nd	
	R_{10}	0·80 (3)‖	−0·36	10 (3)	2·2	nd	
SE (transformed values)			±0·18		±0·2		±0·09
Statistical significance of differences between treatments: P**			> 0·001		> 0·05		> 0·01
Statistical significance of differences between sampling periods: P**			> 0·001		NS		> 0·001

NS, not significant; nd, not determined.

* For details of experimental procedures, see p. 384.

† For details of composition, see Table 1.

‡ A mixture of (g/kg diet) 20 adenosine, 22 guanosine, 17 uridine, 15 cytidine.

§ RNA content by analysis.

‖ Groups containing values below limits of detection; mean value therefore represents an upper limit.

¶ Urine samples collected in periods 0–5 and 0–11 h.

** Statistical analysis (including analysis of variance) done on transformed values (for 5 and 11 h samples only) to accommodate the positive relationship which exists between mean values and their variances (Snedecor & Cochran, 1967).

of plasma and urine are given in Table 8. The amount of phosphate in the digestive tract was increased by dietary RNA (except on day 1), by some 50% in the small intestine, but by at least 300% in the colon. No significant change in plasma inorganic phosphate concentration was detectable at the time of sampling, but the over-all change in phosphate metabolism caused by RNA administration is indicated by the 5-fold increase in urine phosphate in a 24 h period.

The effect of RNA administration on the rate of passage of food along the digestive tract

Since nucleosides tend to reduce gut motility (Kim, Schulman & Levine, 1968), we monitored the passage of food along the digestive tract. The Cr_2O_3 contents of the various sections of the digestive tract of three rats/treatment killed on days 1, 2, 4 and 19 of the 19 d trial period in Expt 4 were determined. Analysis of variance

revealed no detectable difference in the distribution of the marker between the three dietary treatments. The Cr_2O_3 contents of the stomach, small intestine, caecum and colon 5 h after rats were given access to food were (mg/rat; mean and SE); $0{\cdot}62 \pm 0{\cdot}09$, $0{\cdot}31 \pm 0{\cdot}02$, $0{\cdot}38 \pm 0{\cdot}03$ and $0{\cdot}34 \pm 0{\cdot}04$ respectively, suggesting that cardiac blood samples were being taken for analysis at a time when digesta were present in the sections of the gut where RNA metabolism primarily occurs (Wilson, 1962). Fig. 2 shows that in the rats in which food intake was determined, the average food consumption was decreased only slightly by inclusion of RNA in the diet, and only for the first few days.

Expt 5. Changes in ribose levels of digesta, plasma and urine following the incorporation of RNA, nucleosides and ribose into the diet

As expected, inclusion of free ribose in the diet greatly increased the amount subsequently measured in digesta from the small intestine (Table 9). This was not, however, reflected in the composition of plasma. Where RNA and the nucleoside mixture were fed, analysis of variance (evaluation of least significant differences) of results indicated that free ribose concentrations were increased, not only in the intestine, but also in urine.

DISCUSSION

A preparation of 'single-cell' protein which contains 1 g nucleic acids produces on hydrolysis approximately 340, 260 and 400 mg mixed purine and pyrimidine bases, phosphoric acid and pentose sugar respectively. By comparison, the normal daily output of these substances by a 70 kg man is quoted as being approximately 140 mg (largely uric acid), 2650 mg and trace quantities respectively (Altman & Dittmer, 1974).

These values provide some indication of the potential of dietary nucleic acids for altering the composition of body fluids but, of course, take no account of the capacity which exists in many tissues for utilization of the products formed.

In the studies described in this paper, RNA was administered at very high levels. Clearly, therefore, we are dealing with an experimental rather than a practical situation. Nevertheless, a realistic approach was maintained, since values for protein:nucleic acid of 3:1 and even less have been quoted for rapidly growing bacteria (Elsworth *et al.* 1968). In these extreme circumstances, diets containing 300 g/kg protein would, if the latter were derived entirely from 'single-cell' protein, contain as much nucleic acid as any of the diets used in the present study.

There have been a number of reports concerning the changes in urine composition resulting from the oral administration of nucleic acid, usually yeast RNA; both in man (Waslien, Calloway & Margen, 1918; Bowering, Margen, Calloway & Rhyne, 1969; Bowering, Calloway, Margen & Kaufmann, 1970; Edozien, Udo, Young & Scrimshaw, 1970) and other animals (Roll, Brown, Di Carlo & Schultz, 1949; Barnard, 1969; and for review, Rose, 1924). However, most studies have involved the determination of a single degradation product (uric acid), and there have been no studies which include the simultaneous analysis of both blood and digesta.

The results of our studies indicate that uric acid, the main product of nucleic

acid metabolism in man, is easily detectable in the gut, plasma and urine of the rat when the diet contains no RNA supplement. By comparison, the response to oral administration of RNA is quite small though, for instance, a 2·5-fold increase in its urinary excretion was found in animals given diet $R_{10(2)}$ (86 g RNA/kg, replacing cellulose in the control diet) (Table 4). Uric acid formation is not of major importance in the disposal of purine residues, since allantoin is eliminated in considerably larger amounts (Table 7). This was recognized in early work involving oral administration of RNA (for review, Rose, 1924) and is apparent in the results quoted by Roll *et al.* (1949) and Barnard (1969). It also correlates well with the finding that in rats given a bacterial 'single-cell' protein, the most conspicuous response detected by Stringer & Litchfield (1973) was the increase in plasma allantoin concentrations. Our finding that metabolites such as hypoxanthine and xanthine were only present in the gut, appeared to confirm that the earlier stages of purine degradation are not rate-limiting in the rat (cf. Getler, Roll, Tinker & Brown, 1949; Wilson & Wilson, 1962).

Uracil has been detected in both plasma and urine of man (Adams *et al.* 1960; Jolley & Scott, 1970) as well as lymph derived from the thoracic duct of the rat (Dietrich & Siegel, 1960); although there was no deliberate administration of its precursors in the diet. However, the very marked changes in uracil concentrations which were found in the present study to result from RNA administration have not been previously recorded. The response generally (though not universally) attributed to the pyrimidine residues of RNA has been an increased excretion of urea via the kidney, both in man (Frank & Schittenhelm, 1909) and other animals (Wilson, 1923; Cerecedo & Emerson, 1930). In our experiments, uracil accumulation was so high in RNA-fed rats that it was first recognized as an incrustation on the urine channel of metabolism cages (Peers, 1974).

A change similar to that occurring in urine composition was also found in plasma. In the longer-term experiment (Expt 4), uric acid concentrations in blood were not appreciably increased, but uracil concentrations were 20-fold higher than those of the control animals (Table 6).

The sharp increase in plasma uracil might be expected to cause changes in the internal environment of cells around which they circulate, and in this way affect their behaviour. Thus, for instance, there is good evidence that the chief limitation to uracil 'salvage' into the nucleotide pool in many tissues is the availability of uracil rather than of the enzymes required (Canallakis, 1957; Cooper, Dunning & Green, 1972).

In a preliminary study of this possibility, the composition of erythrocyte cytoplasm was compared with that of suspending plasma. Although no precise conclusions could be drawn, the uracil levels in the two media were of the same order under a quite wide range of conditions (Table 3). There have been no reports of studies of transport of uracil into rat erythrocytes. However, the results of studies on other cells and tissues suggest that it occurs fairly freely by a non-gradient-forming mechanism (cf. Jacquez, 1962) and that having entered, uracil is not subsequently transformed to any appreciable extent (Barrett, Munavalli & Newark, 1964; Oliver

& Paterson, 1971). Our findings indicate that in rat erythrocytes this is true of both uracil and uridine. In the latter instance, there is more direct evidence, though it relates to human rather than rat erythrocytes (Lieu, Hudson, Brown & White, 1971; Oliver & Paterson, 1971). With regard to the purines the results were not conclusive. Inosine, xanthine and hypoxanthine, which were just measurable within erythrocytes, could not be detected in plasma. There is no evidence for active transport of any of these substances in erythrocytes at least in the human (Whittam, 1960; Lassen, 1967), although slight concentration gradients might be expected simply as a response to electrical polarization of the membrane. However, while it is clear that there are important interspecies variations, erythrocytes generally have a considerable capacity for interconverting purine bases, their nucleosides and their nucleotides (Bishop, 1964). It is therefore possible that sufficient time elapsed during preparation of the erythrocytes for analysis, for some artifactual nucleoside and free-base accumulation to occur.

The liver has been extensively studied as a site of purine and pyrimidine metabolism. However, it is clear from the studies of Wilson & Wilson (1958) and Wilson & Wilson (1962) that the transformations occurring in the gut extend beyond the release of nucleosides and free bases from nucleic acids by the nucleases, phosphodiesterases and phosphorylases (Wilson, 1962). It is probable, in fact, that these additional reactions are so important as to impose an important influence on the composition of blood and urine.

An interesting finding in our analyses of the water-soluble materials in the gut was the extent to which base and nucleoside accumulation occurred when animals were given the control diet. These substances were obviously products of endogenous metabolism which, at least for purine bases, may include secretion across the wall of the intestine (Berlin & Hawkins, 1968). If, as seems probable, they are derived primarily from the extrusion and degradation of intestinal epithelial cells (Hooper, 1956), their relative concentrations might be expected to reflect the composition of intercellular rather than extracellular fluid (cf. erythrocyte analyses in Table 3).

The accumulation of nucleosides and bases in the gut of control animals complicates the determination of transformations resulting from the presence of exogenous RNA. However, it appears that the changes found in gut composition which occurred when this material was incorporated into the diet were similar to those suggested by the results of Wilson & Wilson (1958) and Wilson & Wilson (1962); adenine and cytosine did not accumulate, while the levels of hypoxanthine, xanthine, uric acid, inosine, uracil and uridine were all increased. Since the increases in these various substances do not resemble their relative abundance in dietary RNA, it is clear that differential transformation (Wilson & Wilson, 1958; Wilson & Wilson, 1962) and absorption (Schanker & Tocco, 1960; Schanker, Jeffrey & Tocco, 1963; Berlin & Hawkins, 1968) are important factors in regulating their accumulation in the gut.

The complex relationship which exists between the purine and pyrimidine derivatives in digesta and those in blood and urine is also found when nucleoside and free base mixtures, rather than nucleic acids, are given to rats. The effects of

nucleosides are, in fact, similar to those caused by dietary RNA, detectable changes occurring only in the amounts of uric acid and uracil. In contrast, the response in body-fluid composition to the administration of equivalent mixtures of bases, was strikingly different. Thus, in addition to uracil, the bases cytosine, adenine and hypoxanthine were also found in urine under these circumstances (cf. Mendel & Myers, 1910; Wilson, 1923; Duell, 1924; Cerecedo, 1927; and for review, Rose, 1924). The presence of hypoxanthine may indicate the importance of the pathway for adenine metabolism not involving direct oxidation to 2,8-dioxyadenine (Bendich, Brown, Philips & Thiersch, 1950).

Wilson & Wilson (1958) found that when uridine was added to media surrounding the mucosal surface of guinea-pig and rat intestine in vitro, slight accumulation of free ribose occurred; presumably reflecting, rather indirectly, the activity of uridine phosphorylase (*EC* 2.4.2.3). The finding that ribose accumulation did not equal that of the associated free base, suggested that extensive pentose phosphorylation and metabolism occurred within the intestinal epithelium. In our experiments, dietary RNA increased both intestinal and urinary ribose accumulation, but had no significant effect on its plasma concentration. Therefore, it would appear unlikely that the amount of free ribose derived from RNA is sufficient to influence body function appreciably, even though this sugar has a hypoglycaemic effect in a number of animals including man (Segal, Foley & Wyngaarden, 1957; Sloviter, Iteka & Sakata, 1964; Hetenyi & Ishiwata, 1968), which is probably the result of its influence on insulin secretion by the pancreas (Steinberg, Ortman, Poucher, Cochran & Gwinup, 1967; Ishiwata, Hetenyi & Vranic, 1969).

Excessive inorganic phosphate accumulation in the intestine is potentially harmful, partly because it can interfere with calcium metabolism (Borle, 1974). In the present study, dietary RNA caused only a slight increase in inorganic phosphate content in the small intestine, but its effect was considerably greater in the colon.

The inorganic phosphate formed in the gut was clearly quite efficiently absorbed into the body, as it was calculated that its intake as RNA could be accounted for almost entirely as the increment in urinary phosphate output. Nevertheless the absence of any detectable change in plasma phosphate concentrations indicates clearly the efficiency of the homoeostatic mechanisms which regulate its distribution within the body.

The rat appeared to utilize most of the products formed from exogenous nucleic acid quite efficiently (though not necessarily if the same substances are derived from other sources). Uracil, however, was a prime exception and its behaviour has therefore been made the subject of a more detailed study (M.-L. Rabouhans and J. I. Davies, unpublished results). There is little evidence that uracil in blood is harmful, or that its accumulation in body fluids can account for our findings concerning the effects of RNA on energy substrate concentrations in blood (Peers *et al.* 1973). The effect of uracil on thyroid function reported by Fillios, Naito, Andrus & Roach (1960), may well be slight, since Maloof & Soodak (1961) were unable to detect it in their experiments with rats maintained on less unusual diets.

In most physiological test systems, the nucleosides have proved to be more potent

effectors than the corresponding free bases. There is not much accumulation in body fluids, except in the gut (Table 5).

The experiments described in this paper relate entirely to the rat, but the results suggest caution when planning large-scale use of 'single-cell' proteins. The findings presumably do not apply to ruminants, which normally metabolize large quantities of nucleic acids (Smith & McAllan, 1971) and may be of only minor relevance in man, where the rate of uric acid formation may be an even more important limiting factor. Nevertheless, the possibility of disturbing uracil metabolism might warrant particular attention where 'single-cell' protein is included in diets for long periods under non-laboratory conditions with less precisely controlled levels of intake.

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