Roberts & Whelan (1951) have shown that this enzyme, when purified, has no action on maltose.

We are not at this stage particularly concerned with differences between 'caries active' and 'caries inactive' groups of subjects. We also realize that the present DMF number is not necessarily an indication of the present caries activity. Nevertheless, certain interesting observations can be made. In group I (low DMF) fructose is metabolized at a significantly lower rate than any of the other sugars. There are also significant differences in the rates of oxidation of maltose and sucrose, and of maltose and glucose. There is no such difference in the case of glucose and sucrose. In group II (high DMF) the only conclusion which can be drawn is that fructose is metabolized at a lesser rate than the other three sugars. Our results therefore suggest that the pattern of utilization of different sugars by group I subjects differs from that of group II subjects. It is, however, unwise to deduce more from these observations on so few subjects.

SUMMARY

1. The salivary flora metabolize fructose at a significantly lower rate than glucose, maltose, or sucrose.

2. Subjects with a comparatively low DMF number (number of teeth decayed, missing or filled) also show differences in oxidation rate between glucose and maltose, and between sucrose and maltose, but not between sucrose and glucose.

3. Subjects with a high DMF number did not show the differences enumerated in paragraph 2.

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The Breakdown of Urea in Cats not Secreting Gastric Juice

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Although Luck (1924*a, b*) had shown urease to be present in the stomachs of a wide variety of animals, urea was until recently regarded as solely an end-product of mammalian nitrogen metabolism. Experiments with [¹⁴C]urea (Leifer, Roth & Hempelmann, 1948; Langham, 1949; Jones, 1950; Hastings, Langham, Carter & Roth, 1950; Skipper *et al.* 1951; Kornberg, Davies & Wood, 1953*a*) and [¹⁵N]urea (Davies & Kornberg, 1950; Von Korff, Ferguson & Glick, 1951; Kornberg & Davies,

1952*a, b*) showed, however, that cats, dogs, mice, and guinea pigs, could hydrolyse urea *in vivo* and that urea was therefore not metabolically inert.

The experiments reported in this and a subsequent paper (Kornberg, Davies & Wood, 1954) were undertaken to study the rate of, and the conditions affecting, the breakdown of [¹⁴C]urea in cats, whose stomachs were allowed either to rest or to secrete gastric juice. Previous investigations of the behaviour in the cat of [¹⁴C]carbon dioxide liberated into the blood (Kornberg, Davies & Wood, 1952*a*) had shown that the amount of [¹⁴C]urea hydrolysed

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in vivo could be determined from the amounts of [^{14}C]carbon dioxide produced and subsequently expired. This procedure was used in the present work. In the earlier studies of urea breakdown in mice (Leifer *et al.* 1948; Jones, 1950; Hastings *et al.* 1950; Skipper *et al.* 1951) and guinea pigs (Langham, 1949), the experimental animals were placed in respiration chambers after injection of [^{14}C]urea, and the quantity of urea hydrolysed was also determined from the quantity of [^{14}C]carbon dioxide expired. This procedure did not allow of any surgical interference with the animals during experiments or the taking of blood and tissue samples, and the present work was therefore carried out with cats attached to a special respiration circuit (Kornberg *et al.* 1952*a*) by a tracheal cannula, leaving the rest of the animal accessible. By this technique, information was obtained on the rate of urea breakdown in the cat, and on the site of this breakdown in the animal.

Part of this work has been communicated to the Biochemical Society (Kornberg, Davies & Wood, 1953*b*).

EXPERIMENTAL AND RESULTS

General. The general experimental techniques used in the treatment of cats, in the collection and analysis of respiratory, blood, and tissue samples, and in the measurement of radioactivity, were those described previously (Kornberg *et al.* 1952*a*, 1953*a*). The special treatments employed in some experiments are described later. All cats were fasted overnight.

Labelled urea. The [^{14}C]urea used, synthesized according to Murray & Ronzio (1949), was obtained in two consignments each of 1 mc. from the National Chemical Laboratory, Teddington, and the Radiochemical Centre, Amersham, respectively.

The first consignment was received as the crude, final reaction mixture. The urea was isolated, after addition of A.R. urea as carrier, by crystallization from propanol, and was purified by recrystallization from isopentanol. The m.p. was 132° (uncorr.), and the mixed m.p. 132° (uncorr.). The urea (415 mg.) was dissolved in 15 ml. of water; 1.5 ml. of this solution (containing 41.5 mg. of urea and giving 2130 000 counts/min. under the conditions of counting employed) were used for any one cat experiment.

The second consignment was received as 27.4 mg. of pure urea. It was dissolved in 15 ml. of water; 1 ml. of this solution (containing 1.83 mg. of urea and giving 2030 000 counts/min.) was used for any one cat experiment. The acid-volatile $^{14}\text{CO}_2$ of the [^{14}C]urea solutions, which were stored at -12°, was determined before each experiment and was always less than 0.005 % of the total radioactivity.

Determination of gastric urease activity. The urease activities of cat gastric mucosae were measured manometrically. The stomach was removed from the cat, opened, and washed rapidly under running, cold water. It was then transferred to a bacteriological inoculating cabinet. The subsequent treatment of the tissue was carried out in this container, using only sterile solutions and sterile instruments, to prevent contamination of the tissue with bacteria.

The mucosa was scraped off with a microscope slide, weighed, and ground with one-tenth of its weight of acid-washed sand and twice its weight of water. Of the resultant suspensions, 1 or 2 ml. were pipetted into the main compartment of a double-armed Warburg vessel, together with 2 or 1 ml. of water and 0.2 ml. of 3*M*-sodium acetate buffer, pH 5.0. Into the first side arm were placed 0.4 ml. of 0.8*M* urea and 0.1 ml. of 3*M* sodium acetate buffer, pH 5.0; into the second side arm was pipetted 0.5 ml. of jack bean urease solution, prepared as described previously (Davies & Kornberg, 1951).

The vessel was attached to a Warburg microrespirometer, the system equilibrated at 38°, and the contents of the first side arm mixed with those of the main compartment. The evolution of gas due to the hydrolysis of urea was measured for 30–45 min., after which time the contents of the second side arm were mixed with those of the main compartment. The immediate evolution of gas at this stage showed that any absence of gas output after the first mixing was due to the absence of urease activity in the mucosa, and not to experimental errors.

Units. The urease activity of the mucosa was expressed as Q_{urea} ($\mu\text{l. CO}_2$ evolved/mg. dry wt./hr., where 22.26 $\mu\text{l. CO}_2$ at s.t.p. are equivalent to 1 μmole of urea).

Assay of bacterial content. Through the kindness of Dr J. K. A. Beverley, of the Department of Bacteriology, University of Sheffield, it was possible to make a bacteriological examination of the samples of gastric mucosa, obtained as described above, and of samples of various tissues removed from the cats throughout the experiments. Small portions of these samples were inoculated into aerobic and anaerobic media, the constitutions of which were as follows. *Aerobic KU medium:* Pronutrin casein hydrolysate (Herts Pharmaceuticals Ltd., Welwyn Garden City), Lab-Lemco beef extract (Oxo Ltd., London), Bacto yeast extract (Difco Laboratories Inc., Detroit), and urea. Each constituent had a final concentration of 1 % (w/v). Samples (20 ml.) of this solution were adjusted to pH 7 with 0.05*N*-NaOH, placed in 500 ml. conical flasks, inoculated and kept at 37° for 48 hr. *Anaerobic KU medium:* a narrow, screw-cap bottle was completely filled with 10 ml. of the aerobic KU medium, described above, and 5 g. cooked, minced ox heart. The inoculated mixture was similarly kept at 37° for 48 hr.

Samples from those liquid cultures in which growth had occurred were inoculated on to plates of the aerobic KU medium solidified with agar and containing in addition 0.02 % of phenol red. These plates were incubated at 37° for 48 hr., aerobically in the case of the aerobic cultures and both aerobically and anaerobically in the case of the anaerobic cultures. The presence of an alkaline zone around a colony indicated that the colony contained urease. Since none of the obligate anaerobes contained urease, the urease-positive colonies were inoculated into 20 ml. of a liquid medium contained in a 250 ml. conical flask and similar in composition to the aerobic KU medium save that it contained no urea. The flasks were incubated aerobically for 48 hr. at 37°.

The urease activities of samples of these cultures and of the bacteria-free supernatants obtained after centrifugation were subsequently measured manometrically. All the cultures grown from the urease-positive colonies contained urease, although urea had been omitted from the final medium in order to reduce the blank in the urease estimation.

The 'urea space' of the cat

When [^{14}C]urea was injected intravenously into cats, the specific activity of the blood urea rose sharply. As the labelled urea mixed with the urea in the rest of the body, the specific activity fell rapidly until, some 30 min. after the injection, the rate of fall lessened. At about 60 min. after the injection, the mixing of labelled and unlabelled urea appeared to be complete and the specific activity of the blood urea after this time decreased only slowly (by about 5%/hr. of the value at 60 min.). This fall in the later stages of the experiments was due to the dilution of the labelled urea with unlabelled urea synthesized in the liver; since the ureters were tied in all experiments, this dilution manifested itself by a steady rise in the concentration of urea in the blood. The results of two typical experiments are given in Table 1. After injection of the labelled urea, the counts/100 ml. of blood fell rapidly until, at approximately 60 min., constant values were reached and maintained for the remainder of the experiments. The radioactivities at these levels

were 194 000 and 108 000 counts/min./100 ml. of blood for the two cats, i.e. 211 000 and 117 000 counts/min./100 ml. of plasma water, respectively. Since in each case 2 130 000 counts/min. had been injected, the labelled urea had thus been diluted with unlabelled urea dissolved in 1010 ml. (cat 1) and 1820 ml. (cat 2) of body water; the urea space of cat 1 (1.57 kg. body weight) therefore was 64.5% of the body weight, and that of cat 2 (2.80 kg. body weight) was 65.0%. These values, previously reported by Kornberg, Davies & Wood (1952*b*), are very similar to those found simultaneously and independently by Eggleton (1951).

The expiration of [^{14}C]carbon dioxide

After intravenous injection of [^{14}C]urea into cats, [^{14}C]carbon dioxide appeared rapidly in the expired air. For the first 30–40 min. after administration of the isotope, the specific activity of the expired carbon dioxide rose sharply, reaching a constant or slightly rising level after this time (Fig. 1). The total amount of [^{14}C]carbon dioxide expired, which varied with the size of the experimental animal,

Table 1. *Changes in the concentration and specific activity of blood urea after intravenous injection of [^{14}C] urea*

(Cat 1; lean male cat, 1.57 kg. body wt.; cat 2; male cat, 2.80 kg. body wt. Ureters tied 5 min. before injection of 2 130 000 counts/min. at 0 hr.)

Cat no.	Time after injection (hr. min.)	Concentration of urea (mg./100 ml. whole blood)	Specific activity of blood urea (counts/min./mg. C)	Radioactivity of whole blood (counts/min./100 ml.)
1	0 0	29.6	0	0
	0 4	39.7	55 700	442 000
	0 31	37.5	28 800	216 000
	0 59	38.8	24 800	192 000
	1 26	38.8	25 500	198 000
	1 49	40.7	24 000	196 000
	2 16	42.2	22 200	187 000
	2 50	46.7	21 300	199 000
	3 21	44.2*	20 400	180 000
	3 48	47.7	19 400	185 000
	4 30	47.7	19 200	183 000
	4 51	51.8	18 000	185 000
2	0 0	35.9	0	0
	0 5	40.7	28 900	235 000
	0 20	40.5	14 100	114 000
	0 35	42.9	12 400	107 000
	0 50	47.4	10 500	99 600
	1 05	49.1	10 700	105 000
	1 20	55.4	9 210	102 000
	1 35	55.2	8 720	96 300
	1 50	57.5	8 800	101 000
	2 10	57.5	9 100	105 000
	2 21	57.7	8 440	97 500
	2 35	59.9	8 440	101 000
	2 50	65.3	8 320	109 000
	3 05	68.8†	7 960	109 000
	3 20	68.8	7 740	107 000
	3 35	68.1	8 200	112 000
	3 50	69.4	7 830	109 000
	4 05	70.3	6 280	88 400

* 20 ml. saline injected at 3 hr. 20 min.

† 15 ml. saline injected at 3 hr.

Table 2. *Urea breakdown in cats not secreting gastric juice*

(In these experiments, carried out on cats whose ureters were tied before injection of [^{14}C]urea at 0 hr., the concentration of urea in the body fluids rose throughout, and the average urea content was taken to be 7 m-moles/l. of body water. The total body water was 65% of the body weight.)

Cat no.	Wt. before injection of [^{14}C]urea (kg.)	Urea contents (m-moles)	Treatment		Rate of urea breakdown ($\mu\text{moles/hr.}$)	
			Before injection of [^{14}C]urea (a)	After 2 hr. (b)	After treatment (a)	After treatment (b)
4	2.70	12	None	Stomach removed	63	64
5	1.55	6.8	Intestine removed from pylorus to anus	Stomach removed	40	40
10	1.15	5.2	None	None	41	43
11	1.96	8.8	All abdominal viscera removed*	None	37	—
12	1.51	6.7	Kidneys, intestine from pylorus to anus, removed	None	24	27
13	1.50	6.7	None	Kidneys removed	56	58
14	1.4	6.4	Kidneys, uterus, ovaries, and gastro-intestinal tract, from cardiac sphincter to anus, removed	None	65	67
22	1.3	5.9	Gastro-intestinal tract, from cardiac sphincter to anus, removed	None	1	—
23	1.6	7.4	Gastro-intestinal tract, from cardiac sphincter to anus, removed	None	37	—
26	—	—	Blood supply left only to heart and lungs	None	<0.5	—
27	—	—	Blood supply left only through head and neck, heart and lungs	None	12	—

* These were the liver, stomach, intestine from pylorus to anus, spleen, pancreas, suprarenals, kidneys, bladder, uterus, and ovaries.

ranged from 0.011 to 0.61% of the total ^{14}C injected/hr., showing that 1–67 μmoles of urea/hr. were hydrolysed in the body (Table 2).

The influence of the viscera on the breakdown of urea

When the stomach was removed 2 hr. after injection of the isotopic material, there was no decrease in the rate of expiration of [^{14}C]carbon dioxide over the next 2 hr. (Fig. 1, curve A). When this experiment was repeated on an animal from which the intestinal tract, from pyloric sphincter to anus, had been removed before the injection of the labelled urea, a similar result was obtained (Fig. 1, curve B). Previous experiments (Kornberg *et al.* 1952*a*) had established that cessation of [^{14}C]carbon dioxide production in the body would be followed by a rapid fall in the specific activity of expired carbon dioxide. These results therefore show that, although suspensions of the gastric mucosae of these three cats were found to have urease activity with Q_{urea} values of 2, 8 and 14, the breakdown of urea in these cats was not due to the activity of gastric urease. They further show (cf. Table 2) that this breakdown was also not due to the action of bacteria in the intestine, but was due to the

Fig. 1. The expiration of ^{14}C after intravenous injection of [^{14}C]urea into fasting, anaesthetized cats with tied ureters. 2130000 counts/min. injected at 0 hr. A, cat 4; B, cat 5; intestine removed from pylorus to anus at 0 hr.; C, cat 11; all abdominal viscera removed at 0 hr. For further details, see Table 2.

occurrence of urea-splitting processes elsewhere in the body.

This failure to abolish the breakdown of urea by removal of the intestine (Fig. 1, curves *B* and *C*; Table 2) was again demonstrated when the gastro-intestinal tract, from cardiac sphincter to anus, was removed some hours after the injection of the urea, and only a slight decrease in the specific activity of the expired carbon dioxide occurred (cf. Kornberg *et al.* 1954). The observed breakdown of urea was therefore not due to the presence, throughout the experiments, of urea-splitting micro-organisms in this part of the intestinal tract, as had been suggested from earlier experiments on the metabolism of [^{15}N]urea in rats (Schoenheimer, 1942; Bloch, 1946).

Curve *C* of Fig. 1 was obtained with a cat (no. 11, Table 2) from which had been removed, before the injection of the urea, the liver, stomach, intestine from pylorus to anus, spleen, pancreas, suprarenals, kidneys, bladder, uterus, and ovaries. Despite this almost complete evisceration, the breakdown of urea occurred at a rate comparable with that found with normal cats, since 37 μmoles of urea were hydrolysed in the first hour. This showed that the organs removed made no major contribution to the breakdown of urea by the normal cat.

Three explanations appeared to be possible to account for this breakdown. (*a*) The rapid removal of ammonia from the blood by the liver might cause the reactions

to move to the right, thus causing a non-enzymic breakdown of urea. (*b*) Urease might be distributed throughout the body but not localized in any one particular tissue. (*c*) Some tissue or tissues not yet identified might contain urease or urea-splitting organisms in quantities sufficient to catalyse the observed hydrolysis of urea.

Physicochemical transformations of urea

Urea in neutral, aqueous solution is in equilibrium chiefly with ammonium, cyanate, bicarbonate, and carbamate, ions. In the body, any ammonia thus arising from urea would be rapidly removed from solution, and any bicarbonate would mix with the total carbon dioxide of the body and would subsequently be expired. It was therefore conceivable that the small amounts of [^{14}C]carbon dioxide expired by cats after intravenous injection of [^{14}C]urea might arise from the disturbance in the body, by the two factors mentioned above, of the normal equilibrium between urea and its decom-

position products. Reactions relevant to this physicochemical decomposition of urea (Wyatt & Kornberg, 1952) provided information from which were calculated (Kornberg, 1953) the amounts of [^{14}C]carbon dioxide which would arise by these non-enzymic mechanisms under the conditions existing in the cats. If the hydrolysis of the cyanate ion were not enzymically catalysed, the total quantity of [^{14}C]carbon dioxide produced by the physicochemical decomposition of the injected [^{14}C]urea would be of the order of 0.004 $\mu\text{moles/hr.}$ instead of the average of 41 μmoles normally produced in that time. The rate of this non-enzymic breakdown of urea thus is too slow, by a factor of 10 000, to account for the observed rates of breakdown.

It has been suggested (Holtham & Schütz, 1948) that there is in blood an enzyme, cyanase, which catalyses the hydrolysis of cyanate. If such an enzyme were to play a part in the above scheme of conversion of urea into carbon dioxide, the rate calculated for this conversion would be increased at most by a factor of about 100, i.e. the total quantity of [^{14}C]carbon dioxide produced in this case would rise to a maximum of approximately 0.5 $\mu\text{moles/hr.}$, which is still only 1 % of the observed average rate.

These calculations therefore show that neither with nor without the intervention of a cyanase can the observed breakdown of urea in cats be due solely to the physicochemical reactions of urea under the present conditions, but that an enzymic cleavage of urea must occur.

The distribution of urease in the tissues

In order to establish the site of the enzyme responsible for the *in vivo* hydrolysis of urea, studies were made of the possible urease activity of various cat tissues.

Samples of brain, duodenum, ileum, kidney, liver, oesophagus, ovary, pituitary and adrenal glands, and skeletal muscle, were removed from cats and, with the previously described precautions against bacterial contamination, were ground with a little sand and twice their weight of water.

Samples of the resultant suspensions were tested for the presence of bacteria: growth of urea-splitting organisms occurred only from the suspensions of kidney, ileum, and oesophagus.

Samples of all tissue suspensions were placed in all-glass aeration units (Kornberg *et al.* 1953*a*), together with unlabelled urea containing added [^{14}C]urea (200 000 counts/min.), and sodium phosphate buffer, pH 7.4, both to final concentrations of 0.01 M. The units were placed in a water bath at 37° and a slow stream of 5% CO_2 in O_2 was bubbled through the suspensions for five 1 hr. periods. The emergent CO_2 was collected in 3N-NaOH and its specific activity assayed.

Although suspensions of gastric mucosa treated in this manner caused the rapid appearance of [^{14}C]carbon dioxide in the emergent gas, no such hydrolysis could be detected with any tissue tested except oesophagus and ileum: the rate of production of [^{14}C]carbon dioxide from the other tissue suspensions was immeasurably small, and the rate of urea breakdown was thus less than $0.0004 \mu\text{moles/hr.}$ These results, together with those reported for the breakdown of urea in partially eviscerated cats, exclude the brain, duodenum, kidneys, liver, ovaries, pituitary and adrenal glands, and skeletal muscle, as the sites of the urease responsible for the *in vivo* hydrolysis of urea.

The localization of urease in the head and neck. The possibility that urea-splitting processes in the head and neck of the cats might be responsible for this hydrolysis of urea, was investigated after connecting the cats to a modification of the usual respiration circuit.

The tracheal cannula was connected to a valve, which in turn was connected both to a respiration pump and to the usual respiration circuit, in such a way that the lungs were inflated by the pump and deflated by the respiration circuit, and the chest could thus be opened without killing the cat. The gastro-intestinal tract from duodenum to rectum was removed in the first experiment, as were also the ovaries, uterus, spleen, pancreas and bladder. The oesophagus was removed from its junction with the mouth downwards to the cardiac sphincter. Ligatures were then tied round the carotid arteries and the jugular veins, three more were tied tightly round the neck, and the head and neck above the ligatures were cut off. The now headless cat had, after intravenous injection of dextran solution (6% (w/v) in 0.9% (w/v) NaCl), a blood pressure of 60 mm. Hg; it maintained this pressure and manifested strong, regular heart beats throughout the experiment. [^{14}C]Urea was injected intravenously and the expired air continuously collected in 15 min. samples over the next 2 hr. 15 min.

The total carbon dioxide expired over this time had a very low specific activity and represented a total of only 0.07% of the total isotope injected. Analysis of the blood taken at the end of the experiment showed that the specific activity of the urea at this time was 18000 counts/min./mg. carbon; the low specific activity of the expired carbon dioxide was therefore due to a greatly reduced rate of urea breakdown in the cat. The rate observed, $6 \mu\text{moles/hr.}$, was less than 15% of the average noted in cats which had not been decapitated.

This experiment was repeated with a cat from which only the head was removed before injection of the isotopic material. The rate of urea breakdown was $4 \mu\text{moles/hr.}$ and was unaffected by subsequent removal of the oesophagus, and of the gastro-intestinal tract. A third cat which, by accident, had not been fasted, was decapitated before injection of [^{14}C]urea. It hydrolysed $17 \mu\text{moles}$ of urea

in the first hour (Fig. 2), at which time the gut, from pyloric sphincter to anus, was removed. The amount of urea broken down over the next hour (15 μmoles) was not significantly lower, confirming that this hydrolysis was not caused by urease in the intestine. When the stomach was removed at 2 hr., the rate of expiration of [^{14}C]carbon dioxide fell rapidly, only 5 μmoles of urea being hydrolysed in the period of the experiment from 2 to 3 hr. Since the stomach contents were found to be strongly acidic and to consist of partly digested food, the high rates of breakdown noted in the early stages of the experiment were due to the activity of gastric urease concomitant with the secretion of gastric acid (cf. Kornberg & Davies, 1952*b*; Kornberg *et al.* 1954). The rapid fall in the rate of urea breakdown after gastrectomy showed that the residual urease activity in the cat was at most 10% of that observed on the average in normal cats.

The ability of the head and neck regions to catalyse the breakdown of urea was demonstrated in an experiment in which the circulation to parts below the thorax was cut off.

Blood from the aorta was passed directly into the inferior vena cava through polythene tubing and an artificial resistance, attached to a mercury manometer, which served to regulate the blood pressure in this external circulation. Both the aorta and the inferior vena cava were tied below the cannulae inserted into them, thus leaving in the animal only the pulmonary circulation, and the systemic circulation to the head and neck.

Fig. 2. Urea breakdown in an acid-secreting, decapitated cat. Female cat, 1.9 kg.; decapitated before injection of [^{14}C]urea (3500000 counts/min.) at 0 hr. Intestinal tract from pylorus to anus removed at 1 hr.; stomach removed at 2 hr. The stomach contents were strongly acidic and contained partly digested food.

When [^{14}C]urea was injected into this preparation, the usual rapid appearance of [^{14}C]carbon dioxide in the expired air occurred. Since the liver of this cat received no blood, no urea synthesis took place, and the concentration of urea in the blood remained constant. A total of 612 counts/min. as [^{14}C]carbon dioxide were liberated/hr. in the cat from [^{14}C]urea in the body fluids which had an activity of 360 counts/min./ml. water. The concentration of urea in the plasma was 7.1 mM. Hence the activity of the urea in the cat was 51 counts/min./ μmole urea, and 12 μmoles of urea/hr. were therefore hydrolysed. This rate of breakdown was somewhat lower than the average normally observed in whole cats (Table 2), but was of the same order of magnitude, and higher than the breakdown occurring in decapitated animals.

These results thus show that nearly all the observed hydrolysis of urea in cats not secreting gastric juice occurs through the action of urease situated in the head and neck region of the animals.

Urease activity of cat gastric mucosa. Of thirteen cats examined, ten showed gastric urease activity *in vitro*, the Q_{urea} ranging from 2 to 41, average 14, a further cat examined had a Q_{urea} of 428, and two apparently normal cats showed no gastric urease activity ($Q_{\text{urea}} < 0.1$). All suspensions manifesting urease activity also contained urea-splitting staphylococci (cf. Kornberg *et al.* 1954). The wide variation in the urease activity manifested *in vivo* by normal cats (Table 2), which would be expected as a consequence of a natural variation in the intensity of infection with urea-splitting micro-organisms, was therefore also seen in the wide variation in the urease activity of gastric mucosal suspensions.

The effect of an antibacterial mixture on the breakdown of urea

On the suggestion of Prof. A. Baird Hastings, of Harvard University, the effect on the urea breakdown of administering an antibacterial mixture was studied. Each dose of this mixture (which will for convenience be referred to as 'PTS mixture') consisted of 500 000 units of penicillin, injected intramuscularly as the sodium salt, and 500 mg. Terramycin (Pfizer Ltd., Folkestone) with 2.5 g. sulphaguanidine, administered orally.

When cats were treated with this 'PTS mixture' (two doses per day) for 4 days, and [^{14}C]urea was injected after this time, the carbon dioxide expired was of very low specific activity, and the total amounts of urea hydrolysed by two cats were only 1 and 3 $\mu\text{moles/hr.}$, respectively. Both the intestinal contents and the faeces were free of viable bacteria.

Manometric experiments showed that 'PTS mixture' at even 10 times the concentration given to the cats did not inhibit the urease activity of gastric mucosal suspensions. It was further shown

(Kornberg *et al.* 1954) that the mixture was without direct effect on gastric urease in the intact cat, but acted by removing urea-splitting micro-organisms from the animals. These findings thus demonstrated that neither *in vitro* nor *in vivo* did the 'PTS mixture' inhibit urease. The abolition of the enzymic hydrolysis of urea in cats by treatment with 'PTS mixture' makes it highly probable that the enzyme responsible for the breakdown of urea in untreated cats is of bacterial origin.

DISCUSSION

The 'urea space' and total body water

Urea has been generally regarded as a substance which is uniformly distributed throughout the fluids of the body, although minor concentration differences have been reported in some tissues. Thus, Myers & Fine (1919), Cockrill (1931), Leopold & Bernhard (1917) and Fremont-Smith, Dailey, Merritt, Carroll & Thomas (1931), reported that the concentration of urea in the cerebrospinal fluid was lower than that of the plasma, and Ralls (1943) found a lower concentration in the blood cells. The concentration of urea in gastric juice is also lower than that in the plasma (Kornberg *et al.* 1954).

The error made in assuming urea to be freely diffusible and uniformly distributed throughout all the body water is, however, quantitatively insignificant, and urea has been used frequently in recent years for the measurement of total body water (Painter, 1940; Steffensen, 1947; McCance & Widdowson, 1951a, b; Eggleton, 1951). It was usually assumed in these experiments that the relatively large amounts of urea administered caused no diuresis and no changes either in the urea clearance or in the rate of formation of urea in the body, which might otherwise vitiate the results.

These assumptions became unnecessary when isotopic urea was used, since the urea was administered in negligibly small quantities. The 'urea space' by these means was found to be 64.5–65 % of the body weight (Kornberg *et al.* 1952a), which is in good agreement with the value of 63 ± 1.6 % reported by Eggleton (1951), who did not use labelled urea. This showed that the assumptions made in the use of unlabelled urea were justified.

The total body water of cats, determined by complete desiccation of the animals, was first measured by Hatai (1917) and found to be 66.7 % of the body weight. Recent experiments with heavy water (Moore, 1946; Edelman, 1953) gave slightly lower results of 62–65 %. All these values are, however, in good agreement with the values found for the 'urea space' of the animals, and it must therefore be concluded that, within the limits of experimental error, the 'urea space' and the total body water are identical.

The in vivo hydrolysis of urea

When [^{14}C]urea was intravenously injected into cats, [^{14}C]carbon dioxide rapidly appeared in the expired air. The breakdown of urea thus observed varied greatly from cat to cat, but was constant within any one experiment (Table 2). In attempts to establish the site of this hydrolysis, most of the viscera were removed from cats without noticeably affecting the breakdown of urea in these animals. This ruled out any major concentration of urease in the liver and kidneys, as had been suggested by FitzGerald (1950). Subsequent experiments in which ground-up tissues were incubated with [^{14}C]urea confirmed that no urea hydrolysis occurred in the muscles and these tissues, which represented the bulk of the viscera.

The removal of the stomach and intestine also had no effect on the breakdown of urea in these non-secreting cats. Since urea-splitting organisms could be isolated from both the stomach and intestine, it was apparent that the circulating urea did not come into effective contact with these sources of urease, nor with the gastric urease which could in most cases be shown to be present in the ground-up gastric mucosae. These findings are explained by the observation that clamping off the blood supply of the stomach (and therefore damaging the stomach) caused a manifestation of gastric urease activity *in vivo* (Kornberg *et al.* 1953*a*), and by the fact that the measured concentrations of urea plus half the ammonia in gastric juice, even with cats treated with 'PTS mixture' (which are devoid of gastric urease activity) was less than half those of the blood (Kornberg, 1953; Kornberg *et al.* 1954). There was thus a 'resistance' to the effective contact of urea in the body fluids and the site of gastric urease. This 'resistance' presumably operated also in reducing the contact of the intestinal flora and the circulating urea to quantitatively insignificant proportions.

When these animals were treated with 'PTS mixture' the enzymic breakdown of urea was abolished. As the 'PTS mixture' had no direct inhibitory action on gastric urease either *in vitro* or *in vivo* (Kornberg *et al.* 1954), it was therefore probable that the urease responsible for the hydrolysis of urea in untreated cats was of bacterial origin. This was supported by the fact that one cat (no. 22, Table 2) hydrolysed urea at only $1\ \mu\text{mole/hr.}$, whilst others treated identically or even more drastically (nos. 5, 11, 14, 23) broke down urea at $37\text{--}67\ \mu\text{moles/hr.}$ This variation would be expected with a variation in the intensity of bacterial infestation. The stomach and intestine, which contain the largest proportion of the bacterial content of the cat, were ruled out as the site of this urease; the remaining bacterially infested sites were the mouth,

trachea, nasopharynx, and oesophagus. Decapitated cats hydrolysed urea at less than 15% of the rates observed on the average in normal animals, whilst the isolated head and neck of a cat could break down urea at a rate of the same order of magnitude as intact cats. These findings show that the site of the urease was in the head and neck.

The comments offered above on the 'resistance' between the circulatory urea and the urease in the gastric mucosa and intestinal tract apply also to the urease in the oesophagus, and, indeed, removal of the oesophagus did not cause any diminution of the rate of urea hydrolysis in the animal. The mouth, trachea, and nasopharynx, however, are richly supplied with urea-splitting micro-organisms, and it has been shown (Cary, 1946) that there is in the intact animal a continual process of hydrolysis of salivary urea. Moreover, the concentrations in the saliva of the urea plus half the ammonia, equal the concentration of urea in the plasma (Hench & Aldrich, 1922), which again shows that a continual splitting of urea takes place in the mouth.

It is therefore concluded that the breakdown of urea in cats not secreting gastric juice is due to the presence of urease of bacterial origin in the head and neck region of the animals, and is probably a result of the continual hydrolysis of urea in the saliva and other secretions of the mouth and respiratory tracts.

SUMMARY

1. When [^{14}C]urea was injected intravenously into anaesthetized cats not secreting gastric juice, whose ureters had been tied, the isotope equilibrated within 1 hr. with the urea of the body. This was found to be dissolved in a volume of water ('urea space') corresponding to an average of 65% of the body weight and equal to the total body water of the cats.

2. Of the [^{14}C]urea injected, amounts were hydrolysed which varied widely between different cats but were constant within any one experiment. The amounts of urea broken down (average $41\ \mu\text{moles/hr.}$) were measured from the quantities of [^{14}C]carbon dioxide produced in the body and subsequently expired.

3. It was calculated that this *in vivo* breakdown of about 0.5%/hr. of the body urea could not be due to physicochemical transformations, but that enzymic cleavage of urea must occur.

4. Removal of the oesophagus or all the abdominal viscera did not diminish the breakdown of urea. Although suspensions of only stomach, ileum, and oesophagus, showed urease activity *in vitro*, the site of the urease responsible for the *in vivo* breakdown was not, therefore, located in the oesophagus or abdominal viscera.

5. Cats whose head and neck had been removed before the injection of [^{14}C]urea, hydrolysed urea at

less than 15% of the average rate normally observed, but the head and neck regions on their own could break down urea at higher rates. Washings of mouth, nasopharynx, and trachea, contained high urease activities.

6. Urease activity was abolished by treating cats with a mixture of penicillin, Terramycin, and sulphaguanidine. This mixture had no direct effect on urease *in vitro* or *in vivo*, but acted by removing urease-containing bacteria from the cats.

7. It is concluded that the enzymic breakdown of urea in cats not secreting gastric juice is caused by urease of bacterial origin, situated almost entirely in the head and neck.

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The Activity and Function of Gastric Urease in the Cat

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The presence of urease in suspensions of the gastric mucosae of dogs, cats, sheep, and bullocks, was first established by Luck (1924). Rigoni (1930), Martin (1932), Cardin (1933), Hollan (1947), Fossel (1947) and Glick (1949) showed the enzyme to be present also in human stomachs. FitzGerald (1946) claimed

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to have found urease activity in the gastric mucosae of frogs, rats and pigs.

Although the enzyme occurs in the ground gastric mucosa of a variety of animal species, the evidence for the *in vivo* activity of gastric urease was slight and often contradictory, and its physiological role was still a matter for conjecture when the present series of investigations was begun. As shown