Biochemical Studies of Toxic Agents

3. THE ISOLATION OF 1- AND 2-NAPHTHYLSULPHURIC ACID AND 1- AND 2-NAPHTHYLGLUCURONIDE FROM THE URINE OF RATS DOSED WITH 1- AND 2-NAPHTHOL

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(*Received* 15 *December* 1950)

The determination of the so-called 'ethereal sulphate content' of urine is a simple analytical procedure, and there are many reports in the literature which show that the administration of phenols to experimental animals is followed by an increased excretion of ethereal sulphates in the urine. On the other hand, because of the difficulties which have usually been encountered, relatively few ethereal sulphates have been separated from urine. For many phenols, therefore, the belief that they are converted to arylsulphuric acids in the body is based solely on the observation that when administered to animals they give rise to an increase in the ethereal sulphate content of the urine.

In the free state many arylsulphuric acids are unstable substances, and those which have been isolated from urine have usually been separated in the form of their alkali-metal salts. The separation of such salts from urine is not easily accomplished, however. In the course of investigations designed to find an alternative method of isolating arylsulphuric acids, Laughland & Young (1942) obtained evidence that these compounds can be separated from urine in the form of their salts with organic bases. At the same time, Barton & Young (1943) developed a simple method of preparing salts of this type, and in a study of the salts formed by a series of arylsulphuric acids with various organic bases they found that *p*-toluidine consistently yielded crystalline derivatives with properties well suited to the characterization of the arylsulphuric acids they were studying. Later work by Laughland & Young (1944) showed that the *p*-bromoaniline salts of a number of arylsulphuric acids have a low solubility in water. One outcome of these investigations was that other workers were led to employ organic bases in the separation and characterization of urinary steroid sulphates (cf. Klyne & Marrian, 1945; Klyne, Schachter & Marrian, 1948; Paterson & Klyne, 1948).

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It has long been known that when 1- and 2-naphthol are administered to dogs these compounds are excreted, in part, as glucuronides (Lesnik & Nencki, 1886). Furthermore, the increased excretion of ethereal sulphates which occurs under these conditions has led to the belief that 1- and 2-naphthol are also excreted in conjugation with sulphuric acid (Mauthner, 1881; Lesnik & Nencki, 1886). The isolation of 1- and 2-naphthylsulphuric acid from urine has not been described, however, and the present work was undertaken in order to determine whether these substances could be separated from the urine of animals dosed with 1- and 2-naphthol. This has now been achieved by the isolation of 1-naphthylsulphuric acid (as its *p*-bromoaniline salt) from the urine of rats dosed with 1-naphthol, and by the isolation of 2-naphthylsulphuric acid (as its *p*-toluidine salt) from the urine of rats dosed with 2-naphthol. In the course of isolating the naphthylsulphuric acids it was also found that 1- and 2-naphthylglucuronide can be separated in the form of their *p*-toluidine salts from the urine of rats dosed with 1- and 2-naphthol, respectively. The free naphthols were also isolated from the urine of the dosed animals.

THE METABOLISM OF 1-NAPHTHOL

General methods. Six male and six female white rats weighing between 180 and 220 g. were used in each experiment. The animals were housed in metabolism cages which permitted the collection of urine separate from the faeces. They were fed once a day for a period of 2 hr. on a diet which consisted of Master Fox Breeding Ration (Toronto Elevators Ltd.), supplemented with fresh milk and brown bread. The urine excreted during the feeding period was not collected. Water was available to the animals at all times.

The 1-naphthol was purified by distillation at reduced pressure and was administered to the rats in the form of a 20% (w/v) solution in corn oil. Solutions of 1-naphthol in corn oil darken on standing and a fresh solution was prepared on each day that the animals were dosed. Each rat was given 0·67 ml. of this solution by injection under the skin of the back immediately after the feeding period on 4 successive days. The collection of urine was begun immediately after the first injection and was continued until 2 days had elapsed

after the last injection. The urine was collected each day and was stored in the refrigerator until the end of the experimental period, and it was then used for the isolation procedures described below. The total amount of 1-naphthol administered in each experiment was 6·4 g.

Isolation of 1-*naphthol from urine*

The urine (pH 6–7) was extracted with peroxide-free ether in a continuous extractor for a total period of 24 hr. The ether extract was evaporated to dryness on a water bath and the residue was sublimed at 65–75° under reduced pressure. The sublimate was crystallized from *cyclo*hexane and yielded an almost colourless crystalline product; m.p. 94–95°; mixed m.p. with 1-naphthol, 94–95° (these and other melting points reported herein are uncorrected). (Found: C, 83·0; H, 5·7. Calc. for $C_{10}H_8O$: C, 83·3; H, 5·6%.)

In three experiments the weights of 1-naphthol isolated from the urine were 0·163, 0·152 and 0·187 g. These amounts represented 2·5, 2·4 and 2·8%, respectively, of the 1-naphthol injected.

Isolation of 1-*naphthylglucuronide from urine*

The urine, after the removal of 1-naphthol by the procedure already described, was adjusted to pH 8–9 by the addition of 20% (w/v) NaOH solution. It was then evaporated at 50–60° under reduced pressure to one-fifth of its original volume of 400–500 ml. The concentrated urine was then transferred to a 250 ml. centrifuge bottle and extracted with seven 100 ml. portions of *n*-butanol. The urine was shaken with each portion of butanol for 15 min. on a mechanical shaker, the mixture was then centrifuged, the butanol layer was withdrawn, and 10 ml. of water were added to the urine to maintain its volume. The butanol extracts were combined and evaporated to dryness at 50–60° under reduced pressure. The residue was dissolved in 50 ml. of water at 60–70° and to the solution at this temperature 2·0 g. of solid *p*-toluidine hydrochloride were added with stirring. The solution was allowed to cool to room temperature and yielded a bulky crystalline precipitate. The mixture was left in the refrigerator overnight, filtered, and the precipitate was dissolved in hot water. Charcoal was added to the solution, which was then heated and filtered. The filtrate was allowed to cool to room temperature and was then left overnight in the refrigerator. The white crystalline product was filtered and dried over P_2O_5 *in vacuo* at room temperature.

Properties of the compound. The product was almost insoluble in cold water, but was quite soluble in hot water. It melted with decomposition at 154–156° with previous darkening. This melting point was not changed when the material was recrystallized several times from water. In order to determine the melting point of the compound an approximate melting point was first obtained in the usual way, and the melting point was then determined with a sample introduced into the bath at a temperature 10° below the approximate melting point. (Found: C, 64·4; H, 6·1; N, 3·1. $C_{23}H_{25}O_7N$ requires C, 64·7; H, 5·9; N, 3·3%.) Neutralization equivalent found, 430; calc. for *p-toluidine* 1-*naphthylglucuronidate*, 427. The compound gave a positive naphthoresorcinol test for glucuronide. It gave a positive test with Benedict's reagent after, but not before, it had been boiled with concentrated hydrochloric acid.

Decomposition of the compound with alkali. 10% (w/v) NaOH (10 ml.) was added to 1·0 g. of the compound. The mixture was stirred well and was extracted with three 30 ml. portions of ether. The ether extracts were combined, dried over anhydrous $CaCl_2$ and filtered. The filtrate was evaporated to dryness and the residue was dissolved in hot 95% ethanol. The solution was heated with charcoal and filtered, and the filtrate was diluted with water. The mixture was allowed to stand in the refrigerator and the precipitate was filtered. The product weighed 0·185 g.; m.p. 45–46°; mixed m.p. with *p*-toluidine, 45–46°.

Decomposition of the compound with acid. 6 N-HCl (10 ml.) was added to 1·0 g. of the compound and the mixture was stirred. The compound dissolved and in a short time a precipitate began to settle out. The mixture was left in the refrigerator for several hours and on filtration it yielded 0·730 g. of crystalline product. This was recrystallized from water; m.p. 198–199°; $[\alpha]_D^{20°}$ – 85° in ethanol (*c*, 1). (Found: C, 55·2; H, 5·6. Calc. for $C_{16}H_{16}O_7 . 1·5\ H_2O$: C, 55·3; H, 5·5%.) Neutralization equivalent found, 350; calc. for 1-naphthylglucuronide, 347. Lesnik & Nencki (1886) reported that the 1-naphthylglucuronide, which they isolated from the urine of animals dosed with 1-naphthol, melted at 202–203°.

The compound (1 g.) obtained by decomposition of the *p*-toluidine salt with acid was heated under reflux on a boiling-water bath with 20 ml. of N-H_2SO_4 for 6 hr. The hydrolysate was cooled, left overnight in the refrigerator, and the precipitate was separated by filtration. The filtrate was extracted with three 30 ml. portions of ether, the combined ether extracts were dried over anhydrous $CaCl_2$, filtered and evaporated to dryness. The residue, together with the precipitate from the hydrolysate, was sublimed at about 80° under reduced pressure. This yielded 0·265 g. of sublimate, m.p. 93–94°. When this material was mixed with 1-naphthol, the melting point was not depressed.

Amounts of compound isolated. The amounts of *p*-toluidine 1-naphthylglucuronidate isolated from the urine were 2·8, 2·9 and 3·2 g., and these corresponded to 14·7, 15·2 and 16·8%, respectively, of the 1-naphthol administered.

Isolation of 1-*naphthylsulphuric acid from urine*

The urine collected from twelve rats which had received a total of 6·4 g. of 1-naphthol by subcutaneous injection was extracted with ether and with *n*-butanol by the methods already described. The combined butanol extracts were evaporated to dryness at 50–60° under reduced pressure. The residue obtained on evaporation of the butanol was dissolved in 60 ml. of water at 60–70° and 2·0 g. of *p*-bromoaniline hydrochloride were added with stirring. The mixture was left overnight in the refrigerator and was then filtered. The precipitate, *P*-1, consisted chiefly of *p*-bromoaniline 1-naphthylglucuronidate and it gave a slightly positive test for ethereal sulphate. The filtrate, *F*-1, gave a strongly positive test for ethereal sulphate. *P*-1 was dissolved in hot water, and the hot solution was treated with charcoal and filtered. The filtrate obtained was left in the refrigerator and

was then filtered. The precipitate, *P*-2, gave no test for ethereal sulphate, whereas the filtrate, *F*-2, gave tests for the presence of ethereal sulphate and glucuronidate. *F*-1 and *F*-2 were combined and evaporated to 40 ml. under reduced pressure. The concentrate was left in the refrigerator and when filtered it yielded a precipitate, *P*-3, which consisted of glucuronidate. The filtrate, *F*-3, obtained after separation of *P*-3, was evaporated to dryness at 50–60° under reduced pressure. The residue obtained after evaporation of *F*-3 was extracted with two 20 ml. portions of boiling ethanol, and the ethanol extracts were cooled and filtered. The precipitate, *P*-4, contained no ethereal sulphate. The filtrate, *F*-4, was evaporated to dryness at 50–60° under reduced pressure. The residue was dissolved in the minimum amount of hot water, and the solution was heated with charcoal and filtered through a heated funnel. The filtrate was cooled in the refrigerator and filtered. It yielded a precipitate which consisted of *p*-bromoaniline 1-naphthylsulphate contaminated with *p*-bromoaniline 1-naphthylglucuronidate. The precipitate was recrystallized three times from water and a product free from glucuronidate was obtained.

Properties of the compound. The compound gave positive tests for sulphate and for naphthol after it had been hydrolysed with hydrochloric acid, but not before it had been hydrolysed. (Found: C, 48·5; H, 3·7; N, 3·3. Calc. for $C_{16}H_{14}O_4NSBr$: C, 48·5; H, 3·5; N, 3·5%.) Neutralization equivalent found, 393; calc. for *p*-bromoaniline 1-naphthylsulphate, 396.

Potassium 1-naphthylsulphate was synthesized by the method of Feigenbaum & Neuberg (1941), and 1·0 g. of this compound was converted to the *p*-bromoaniline salt by a procedure similar to that described by Barton & Young (1943). This yielded 0·820 g. of purified product. (Found: C, 48·6; H, 3·6; N, 3·4. Calc. for $C_{16}H_{14}O_4NSBr$: C, 48·5; H, 3·5; N, 3·5%.) The melting point of this compound was ill-defined, as also was the melting point of the *p*-bromoaniline salt of the ethereal sulphate isolated from urine (cf. Laughland & Young, 1944).

Hydrolysis of the compound. 6N-HCl (5 ml.) was added to 0·100 g. of the compound obtained from urine and the mixture was heated in a boiling-water bath for 30 min. The hydrolysate was allowed to cool to room temperature and was then extracted with three 30 ml. portions of ether. The ether extracts were combined, washed with water and dried over anhydrous $CaCl_2$. The ether solution was filtered, the filtrate was evaporated to dryness and the residue was sublimed under reduced pressure. The sublimate, 0·024 g., melted at 94–95°, and the melting point was not depressed when the compound was mixed with 1-naphthol.

Amounts of the compound isolated. In three experiments the amounts of *p*-bromoaniline 1-naphthylsulphate isolated from the urine of the dosed animals were 0·063, 0·087 and 0·098 g., and these corresponded to 0·4, 0·5 and 0·6%, respectively, of the 1-naphthol administered.

THE METABOLISM OF 2-NAPHTHOL

General methods. These were similar to those employed in studying the metabolism of 1-naphthol, except that 2-naphthol was administered as a 12·5% (w/v) solution in corn oil. Each rat received 1·0 ml. of this solution by subcutaneous injection on each of the first 2 days of the experiment and 0·5 ml. of the solution on each of the next 2 days. The total amount of 2-naphthol administered to the group of twelve rats used in each experiment was 4·5 g.

Isolation of 2-naphthol from urine

The urine (pH 6–7) was extracted in a continuous extractor with peroxide-free ether for a total period of 24 hr. The residue obtained on evaporation of the ether extract was dissolved in hot water and the solution was heated with charcoal. The charcoal was removed by filtration and the filtrate was left overnight in the refrigerator. It yielded a colourless crystalline precipitate, m.p. 121–122°, mixed m.p. with 2-naphthol 121–122°. (Found: C, 83·2; H, 5·7. Calc. for $C_{10}H_8O$: C, 83·3; H, 5·6%.)

In two experiments the amounts of 2-naphthol isolated from the urine were 0·404 and 0·610 g. These amounts represented 9·0 and 13·5%, respectively, of the 2-naphthol administered.

Isolation of 2-naphthylglucuronide from urine

2-Naphthylglucuronide was separated from urine in the form of its *p*-toluidine salt by the same procedure as was used for the isolation of 1-naphthylglucuronide.

Properties of the compound. The *p*-toluidine salts of 1- and 2-naphthylglucuronide showed similar properties. The p-*toluidine 2-naphthylglucuronidate* melted at 184–186°. (Found: C, 64·8; H, 5·7; N, 3·2; neutralization equivalent, 429. $C_{23}H_{25}O_7N$ requires C, 64·7; H, 5·9; N, 3·3%; neutralization equivalent, 427.) The compound gave a positive naphthoresorcinol test for glucuronide. It reduced Benedict's reagent after, but not before, it had been hydrolysed with concentrated hydrochloric acid.

Decomposition of the compound with alkali. When 1·0 g. of the compound was decomposed with alkali by the procedure already described for the alkaline decomposition of *p*-toluidine 1-naphthylglucuronidate, 0·182 g. of *p*-toluidine, m.p. and mixed m.p. 45–46°, was obtained.

Decomposition of the compound with acid. When 10 ml. of 6N-HCl were added, with stirring, to 1·0 g. of the compound, a solution was obtained which on standing yielded a precipitate. The mixture was left in the refrigerator for several hours and the precipitate was then separated by filtration. The precipitate, after prolonged drying over P_2O_5 *in vacuo*, weighed 0·610 g., m.p. 149–150°, $[\alpha]_D^{22°}$ – 97° in ethanol (*c*, 1%). (Found: C, 59·9; H, 5·3. Calc. for $C_{16}H_{16}O_7$: C, 60·0; H, 5·0%.) The anhydrous 2-naphthylglucuronide obtained by Lesnik & Nencki (1886), from the urine of dogs dosed with 2-naphthol, melted at 150°.

The compound (1 g.) prepared by the acid decomposition of the *p*-toluidine salt by the method just described was refluxed with 20 ml. of N-H_2SO_4 for 6 hr. The hydrolysate was left in the refrigerator overnight and the precipitate which formed was removed by filtration. The filtrate was extracted with three 30 ml. portions of ether and the combined ether extracts were dried over anhydrous $CaCl_2$ and filtered. The ether solution was evaporated and the residue, together with the precipitate obtained after hydrolysis, was sublimed under reduced pressure. The sublimate weighed 0·312 g.; m.p. 120–121°; mixed m.p. with 2-naphthol, 120–121°.

Amounts of the compound isolated. The amounts of the *p*-toluidine salt of 2-naphthylglucuronide isolated in three experiments were 2·4, 2·5 and 3·0 g., and these corresponded to 18·0, 18·7 and 22·5%, respectively, of the 2-naphthol administered.

Isolation of 2-naphthylsulphuric acid from urine

The procedure used for the isolation of 2-naphthylsulphuric acid from urine resembled closely that already described for the isolation of 1-naphthylsulphuric acid, except that the compound was isolated in the form of the *p*-toluidine salt instead of the *p*-bromoaniline salt.

Properties of the compound. The compound obtained from urine gave positive tests for sulphate and for naphthol after, but not before, it had been hydrolysed with hydrochloric acid. It melted at 156–158° with decomposition, and the mixed melting point with synthetic *p-toluidine 2-naphthylsulphate* was not depressed. (Found: C, 61·7; H, 5·3; N, 4·0. $C_{17}H_{17}O_4NS$ requires C, 61·6; H, 5·1; N, 4·2%.) Neutralization equivalent found, 327; calc. for *p*-toluidine 2-naphthylsulphate, 331.

Potassium 2-naphthylsulphate was synthesized by the method of Feigenbaum & Neuberg (1941), and 1·0 g. of this compound was converted to the *p*-toluidine salt (0·745 g.) by the method of Barton & Young (1943). The product melted with decomposition at 156–158°. (Found: C, 61·8; H, 5·3; N, 4·3. Calc. for $C_{17}H_{17}O_4NS$: C, 61·6; H, 5·1; N, 4·2%.)

Hydrolysis of the compound. The compound (0·100 g.) obtained from urine was heated in a boiling-water bath with 5 ml. of 6N-HCl for 30 min. The solution was left in the refrigerator for 3 hr., and the precipitate which formed was filtered and crystallized from water. The product, 0·028 g., melted at 121–122°, and the mixed melting point with 2-naphthol was not depressed.

Amounts of the compound isolated. The amounts of *p*-toluidine 2-naphthylsulphate isolated from the urine in three experiments were 0·165, 0·187 and 0·204 g. These quantities were derived from 1·6, 1·8 and 2·0%, respectively, of the 2-naphthol injected.

DISCUSSION

Although it has long been accepted that excretion of 1- and 2-naphthylsulphuric acid takes place following the administration of 1- and 2-naphthol, respectively, the isolation of the conjugated compounds from urine does not appear to have been described. The isolation of 1- and 2-naphthylsulphuric acid from the urine of rats dosed with the corresponding naphthols has now been carried out by making use of the fact that salts of arylsulphuric acids react in aqueous solution with the hydrochlorides of organic bases such as *p*-toluidine (Barton & Young, 1943) and *p*-bromoaniline (Laughland & Young, 1944) to give derivatives of the type $[ROSO_3]^-$ $[R'NH_3]^+$. These derivatives are usually crystalline solids with a low solubility in water. In the present work 1- and 2-naphthylglucuronide were also found to form derivatives with organic bases, and by making use of this finding, 14·7–16·8% of the 1-naphthol and 18·0–22·5% of the 2-naphthol administered were recovered from the urine in the form of the *p*-toluidine salts of the corresponding glucuronides. The amounts of the naphthylsulphuric acids obtained from urine in the form of their organic base salts were quite small, and they represented only 0·4–0·6% of the 1-naphthol and 1·6–2·0% of the 2-naphthol administered. The amounts of the conjugated compounds obtained give little indication of the amounts excreted in the urine, however, because of the losses which occurred during the isolation processes. It seems probable, nevertheless, that the naphthylglucuronides were present in the urine in greater amounts than the naphthylsulphuric acids, for in a recent investigation (Young, Morrison & Billett, 1950) in which rats each received a single dose of 0·1 g. of 1- or 2-naphthol by subcutaneous injection, it was found that in the 3-day period after dosing the increase in urinary ethereal sulphate and glucuronide corresponded respectively to 21 and 53% of the 1-naphthol, and 20 and 56% of the 2-naphthol administered. It is noteworthy that Lesnik & Nencki (1886) reported that 1- and 2-naphthol are excreted mainly as glucuronides by the dog.

It was found that 1- and 2-naphthol were excreted by the dosed animals in the present work, for 2·4–2·8% of the 1-naphthol and 9·0–13·5% of the 2-naphthol were recovered unchanged from the urine. As with the conjugated naphthols, however, the object of the experiments was the separation of the compounds in pure form for identification purposes, and the quantities obtained do not give a true picture of the amounts of the compounds present in the urine.

SUMMARY

1. A study has been made of the metabolism of 1- and 2-naphthol following their administration to rats by subcutaneous injection.

2. The excretion of 1- and 2-naphthol in the urine of rats dosed with these compounds has been shown to occur.

3. 1-Naphthylsulphuric acid (as its *p*-bromoaniline salt) and 1-naphthylglucuronide (as its *p*-toluidine salt) have been isolated from the urine of rats dosed with 1-naphthol.

4. 2-Naphthylsulphuric acid and 2-naphthylglucuronide have been isolated in the form of their *p*-toluidine salts from the urine of rats dosed with 2-naphthol.

All the elementary micro-analyses reported above were carried out by Mr Michael Edson. An account of the work described in this paper formed part of a thesis presented to the University of Toronto (Berenbom, 1947).

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Chemical and Electrical Energy Relations for the Stomach

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(*Received* 23 *September* 1950)

The use of isolated acid-secreting gastric mucosa as experimental material makes it possible to measure concomitantly the rate of respiration (a process which makes energy available), and the rate of acid secretion (a process which requires energy). Moreover, the maximum energy that can be made available from respiration, and the minimum energy required to maintain the secretion, can be calculated. The efficiency of energy conversion can therefore be calculated by comparing the ratio of the rate of acid secretion to the rate of respiration found experimentally with the ratio calculated on the assumption that all the energy from respiration is available to do secretory work. This comparison indicates that the secretory process has a very high efficiency: it approaches 100%.

Similarly, the electrical work done by isolated gastric mucosa can be measured and compared with that available from exergonic processes in the tissue. In addition to this, the passage of an electric current through an acid-secreting gastric mucosa can increase or decrease the rate of acid secretion (Rehm, 1945; Rehm & Hokin, 1948; Crane, Davies & Longmuir, 1948*a*, *b*), and estimations can be made of the efficiency of these processes.

A part of this work was communicated to the Biochemical Society, on 24 September 1948 (Crane & Davies, 1948*a*, *b*).

EXPERIMENTAL

Saline media. The salt solutions were those described by Davies & Terner (1949). For manometric experiments the phosphate saline gassed with 100% O_2 was used; for the electrical experiments the bicarbonate saline, gassed with 5% CO_2 + 95% O_2, was used. Both saline solutions contained 0·2% (w/v) glucose.

Preparation of gastric mucosa. Frogs (*Rana temporaria temporaria* L.) were captured locally, housed in an outdoor froggery under natural conditions and provided with an excess of worms and insects for food (Bradford, Crane & Davies, 1950).

The gastric mucosa was washed and isolated immediately after the frog was pithed. For the manometric experiments it was then tied with silk at both ends and weighed (Davies, 1948*a*). For electrical experiments it was opened along the lesser curvature and mounted in a Perspex holder (Crane *et al.* 1948*a*).

Manometric experiments. The Q_{O_2} (μl./mg. dry wt./hr.) of the tied bags of frog gastric mucosa was measured at 25·0° with Warburg manometers in the usual way (Dixon, 1943). Conical cups (20–30 ml. capacity) were used and the CO_2 absorbed by alkali in the centre well. After a 20 min. equilibration period the pressure changes in the manometer were measured for 2–7 hr. Histamine was added from the side arm, when required, to a final concentration of 5×10^{-5} M, after the basal O_2 uptake had become steady. This was usually 1·5–2·5 hr. after the cups had been put into the bath.

At the end of the incubation the swollen bags of mucosa were removed, and the amounts of HCl produced were determined by weighing and by electrometric titration (Davies, 1948*a*; Davies & Longmuir, 1948).

Electrical experiments. The material, apparatus and methods used for the electrical experiments were similar to those previously described by Crane *et al.* (1948*a*, *b*). The Perspex holder containing the frog gastric mucosa was mounted between two chambers at 25·0°. The Q_{HCl} (μl./mg. dry wt./hr.) of the mucosa was estimated by measuring the changes in pH of the secretory solution *in situ*, and also by replacing the secretory solution and estimating its acid content either manometrically by the addition of a bicarbonate solution or by electrometric titration.

The potential difference (p.d.) across the mucosa was measured by a battery-operated Marconi pH meter and potentiometer Type TF 511 C. Saturated calomel electrodes and saturated KCl bridges with internal ground-glass joints, which dipped into the nutrient and secretory solutions, were