view of Morgan's (1944) statement that the A substance from hog mucin was shown by the method of Consden, Gordon & Martin (1944) to contain no fewer than fifteen amino-acids as part of the complex, it is not difficult to understand how changes in the number and kind of these could give rise to differences in serological specificity even as profound as those existing between the A, B and O groups. Although our present knowledge of these substances is very scanty, it is tempting to speculate that they will contain a common or closely related relatively stable polysaccharide constituent. It has been of interest and gratification to find that the two samples of undegraded and highly purified A substances from commercial hog mucin provided for us by Dr Morgan gave rise to the same methylated stable carbohydrate residue with a *l*-fucose 'end-residue' as was obtained from the pepsin A substance (Bray *et al.* 1946). These studies will be continued in collaboration with Dr W. T. J. Morgan.

SUMMARY

1. A method for preparing polysaccharides from gastric contents of individuals of blood groups A, B and O is described. This material is suitable for preliminary chemical studies of a stable carbohydrate constituent.

2. Although partially degraded, the material from A and B group individuals displays considerable activity in inhibiting isoagglutination.

3. The properties of various fractions are described, the significance of the results is discussed, and attention drawn to the complexity of structural problems in this field.

The thanks of the authors are due to Dr W. T. J. Morgan for providing them with generous samples of a blood group A specific substance from hog gastric mucin and to Dr W. Whitelaw and Miss H. Traught of the Dudley Road Hospital, Birmingham, for supplying large amounts of human gastric mucin obtained in the course of test meal investigations.

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# The Fate of Certain Organic Acids and Amides in the Rabbit

## 1. BENZOIC AND PHENYLACETIC ACIDS AND THEIR AMIDES

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During an investigation of the fate of aromatic amino compounds in the rabbit it was observed that in some cases there was a considerable difference between the products of metabolism of an acid and its amide. Thus it was found that, whereas 45 % of a dose of anthranilic acid given to a rabbit could be recovered from its urine (after hydrolysis), only a very small amount could be obtained after giving an equivalent dose of *o*-aminobenzamide. Further, less phenolic material, as judged by the ferric chloride reaction appears to be formed from the acid than from the amide. Since there is little record in the literature of the investigation of such differences, we have planned a series of comparative studies of certain acids and their amides in order to ascertain in what instances and to what extent an acid and its amide may be regarded as metabolically interchangeable.

There are three main aspects of the problem which may be studied: (*a*) the hydrolysis of the amide group, (*b*) the modification of groups already present in the molecule and (*c*) the introduction of hydroxyl or other groups. The differences may, however, be quantitative rather than qualitative, and it is from the former standpoint that these investigations will in the main be conducted.

This paper deals with benzoic and phenylacetic acids and their amides. The detoxication of the two acids has been studied in great detail by many workers. In the rabbit and the dog both are conjugated, partly with glycine (to form hippuric and phenaceturic acids, respectively), and partly with glucuronic acid, the proportions of the possible conjugates varying in different species. In the fowl phenylacetic acid is conjugated with ornithine, and in man and the chimpanzee with glutamine. The fate of benzamide has not been investigated in much detail. Nencki (1873) isolated hippuric acid from human urine after the ingestion of benzamide, suggesting that in man it is hydrolyzed to benzoic acid, and Salkowski (1877) found that the same occurs in the rabbit. Baumann & Herter (1877) claimed that in the dog benzamide is excreted largely unchanged and observed a slight increase in ethereal sulphate excretion. Gonnermann (1902) investigated the action of enzymes upon various amides and anilides, including benzamide, which he found to be hydrolyzed by sheep's liver and kidney. The detoxication of phenylacetamide does not appear to have been investigated, although Nebelthau (1895) refers to its pharmacological action and that of benzamide.

In the work reported here we have studied the formation of hippuric and phenaceturic acids, the ethereal sulphate excretion and the increase in the excretion of reducing substance after administering the four substances under investigation. Rabbits were maintained on a constant diet until analysis showed that their daily excretion of the substances being estimated was as steady as possible. Doses of the acids or amides were then given and the appropriate analyses performed on the urine passed during the subsequent 24 hr.

## METHODS

*Diet and feeding.* The diet adopted consisted of 100 g. cabbage and 50 g. bran (or alternatively 25 g. each of bran and oats) daily, together with water *ad libitum*. The rabbits (does of approximately 2 kg. weight) were housed in metabolism cages fitted into large funnels for the collection of urine. It was necessary to keep the rabbits on the diet for at least 3 weeks before the normal excretion was steady enough for the experiment to be started. The substances being investigated were then given by stomach tube, benzoic acid as its sodium salt, phenylacetic acid in sodium bicarbonate solution and the amides as suspensions in water. The interval between doses was usually a week, but never less than 3 days.

*Estimation of hippuric and phenaceturic acids.* Failure in obtaining consistent results by Quick's formol titration method (1926) or by Weichselbaum & Probstein's precipitation method (1938–9) led us to adopt a method similar to that of Kanzaki (1932). Urine (20 ml.) acidified with 2N-$H_2SO_4$ (1 ml.) was continuously extracted with ether for 3 hr. The ether extract was then evaporated to dryness and then either titrated directly with 0·1N-alkali, or dissolved in 0·1N-alkali by gently warming on a water-bath, made up to 25 ml. and measured volumes titrated with 0·1N-$H_2SO_4$, thymol blue being used as indicator. (The indirect method was more convenient when the extract was crystalline: the procedure adopted did not affect the result.) The ether-soluble acid of normal urine was expressed as hippuric acid, and after dosing the 'extra' acid as hippuric or phenaceturic acid, as appropriate.

Control experiments, in which hippuric or phenaceturic acid added to normal rabbit urine was estimated as above, showed that the method is capable of giving results within 3% of the true value. The percentage recovery of hippuric acid was 98–99% and of phenaceturic acid 99–103%. Further experiments showed that the amount of sulphuric acid extracted by ether from aqueous solutions under the conditions described is negligible, and that warming the ether-soluble material with 0·1N-alkali for up to 15 min. does not affect the titration values. Treatment of benzamide and phenylacetamide with 0·1N-alkali does, in fact, cause some hydrolysis (4% of benzamide and 20% of phenylacetamide were hydrolyzed, as calculated from the amount of ammonia formed), but under the conditions employed there is not sufficient loss of ammonia to make any significant difference to the titratable acidity. Thus, even in the presence of unchanged amide, the results obtained would not be affected.

*Estimation of ethereal sulphate.* This was taken as the difference between total and inorganic sulphate as determined by Folin's method (1905–6).

*Estimation of reducing substances.* The reducing power of the urine was determined using the Shaffer-Hartmann reagent described by Peters & Van Slyke (1932). The estimation was carried out by heating together reagent (10 ml.) and urine (10 ml.) in a boiling tube in a boiling water-bath for 15 min., cooling, acidifying with 5N-$H_2SO_4$ (5 ml.), and titrating the iodine liberated with 0·1N-thiosulphate. Calibration curves for the reagent were constructed using both glucose and glucuronic acid (as veratroyl glucuronide), and it was found that over the range of reducing values that can be estimated under our conditions the ratio of the reducing powers glucose : glucuronic acid was 1·08, the theoretical value.

It was assumed that the increase in the reducing value of the urine caused by dosage was due solely to glucuronic acid in the form of an ester-type glucuronide. There is the theoretical possibility of the introduction into the molecule of a hydroxyl group, which might then be conjugated with glucuronic acid. This glucuronide would be of the non-reducing ether type and it would be necessary to hydrolyze it to liberate glucuronic acid before estimation by the Shaffer-Hartmann method. There is, however, the complication that when normal rabbit urine is hydrolyzed (e.g. by treatment with 1/10 vol. conc. HCl in a boiling water-bath for 90 min.) there is a considerable increase in its reducing value (average 26%). Experiments in which urine from rabbits dosed with the substances under investigation was hydrolyzed showed that the increase in reducing value was not significantly greater than the increases obtained with normal urine. We conclude, therefore, that if any ether-type glucuronide is formed, the amount is too small to be detected with certainty by our method.

## RESULTS

*Normal ether-soluble acid excretion.* This was established for seven rabbits by frequent analysis

of 24 hr. specimens of urine. While the variations in the day-to-day values were from 40 to 1010 mg. (as hippuric acid), the averages over periods of several days ranged from 306 to 553 mg.

The actual nature of the normal ether-soluble acid was not investigated, save to show that extraction with boiling light petroleum (b.p. 40–60°) removed 10–25 % of the acidic material, this amount being equivalent to 50–100 mg. benzoic acid. This is of the same order as the amount of free benzoic acid in rabbit urine found by Raiziss & Dubin (1915).

*Ether-soluble acid excretion after doses of benzoic acid and benzamide.* The doses given were 2 g. sodium benzoate (equivalent to 1·7 g. benzoic acid) and 1·5 or 2 g. benzamide. At this dose level the former was not at all toxic, but benzamide appeared to have a slight narcotic action (cf. Nebelthau, 1895). The 'extra' ether-soluble acid, expressed as hippuric acid, excreted during 24 hr. immediately after the doses is shown in Table 1. In every case the excretion of ether-soluble acid returned to within the normal range within 24 hr. of the dose.

Table 1. *Excretion of 'extra' ether-soluble acid (calculated as hippuric acid) after doses of benzoic acid and benzamide*

| Substance | Rabbit no. | Dose (g.) | 'Extra' acid excreted in 24 hr. (g.) | 'Extra' acid per g. benzoic acid or benzamide given (g.) | Percentage of dose excreted as ether-soluble acid in 24 hr. after dose |
|---|---|---|---|---|---|
| Benzoic acid | 83 | 1·7 | 1·22 | 0·72 | (49) |
| | 92 | 2·0 | 2·00 | 1·00 | 68 |
| | | 2·0 | 2·64 | 1·32 | 90 |
| | | 2·1 | 2·70 | 1·27 | 86 |
| | | 1·7 | 1·53 | 0·90 | 61 |
| | | 1·7 | 2·47 | 1·46 | (99) |
| | 91 | 1·7 | 2·20 | 1·30 | 89 |
| | 99 | 1·7 | 1·79 | 1·05 | 72[d] |
| | 116 | 1·7 | 1·86 | 1·10 | 75[a] |
| | | | | Average | 77 |
| Benzamide | 83 | 1·5 | 0·94 | 0·62 | (42) |
| | | 1·5 | 1·38 | 0·91 | 62 |
| | | 1·5 | 1·73 | 1·14 | 77 |
| | 92 | 1·5 | 1·80 | 1·19 | 81 |
| | | 1·5 | 1·89 | 1·25 | 85 |
| | | 2·0 | 2·10 | 1·04 | 71 |
| | 91 | 1·5 | 1·68 | 1·12 | 76 |
| | 99 | 1·5 | 1·84 | 1·21 | 83[c] |
| | 116 | 1·5 | 1·63 | 1·08 | 73[b] |
| | | | | Average | 76 |

*Notes:*

(i) The figures in brackets are not included in the averages.

(ii) The base-lines used for the calculations are the averages of all normal values obtained. A probable explanation of very high and low values is that the *actual* base-line for the day of the experiment was towards the high or low extreme respectively of the base-line range.

(iii) The results in Tables 1, 2 and 4 marked with similar signs (*a–h*) were obtained in the same experiment.

An estimate of the amount of free benzoic acid excreted was made by extracting the ether-soluble extract with light petroleum, in which benzoic acid is soluble but hippuric acid is not, and titrating with NaOH as before. The amount of acidic material removed was very little greater than that which could be extracted from the normal ether-soluble acid, indicating that the excretion of free benzoic acid is very small, usually less than 0·5 % of the dose. Tulane & Lewis (1933) found that rabbits excreted 0·4–1·6 % of a dose of benzoic acid in an unconjugated form in 6 hr.

Hippuric acid could be isolated in good yield by ether extraction of the acidified urine passed after the administration of benzamide.

*Ether-soluble acid excretion after doses of phenylacetic acid and phenylacetamide.* In general the doses given were 1·5 g. of either the acid or the amide. Phenylacetic acid had no apparent toxic action. In some cases, however, phenylacetamide caused paralysis of the hind limbs which lasted for several hours, but from which the rabbit recovered completely (cf. Nebelthau, 1895). The results obtained are given in Table 2.

In most instances the excretion of 'extra' ether-soluble acid did not appear to be complete within 24 hr., for in these cases the amount excreted during the second day was outside the normal range to an extent which would account for a further 5–39 % of the dose. Tulane & Lewis (1933) state that phenaceturic acid is excreted more slowly than hippuric acid and that the excretion may be prolonged for several days. In general agreement with this is our finding that while the average percentage of a dose of benzoic acid excreted as ether-soluble acid (assumed to be hippuric acid) in 24 hr. is 77, that of phenylacetic acid appearing as phenaceturic acid in the same time is only 53. The results of experiments in which excretion appeared to be complete in the first 24 hr. do not preclude the possibility that further small amounts of phenaceturic acid may be excreted during subsequent days, as these may not raise the excretion of ether-soluble acid above the upper level of the normal range.

As in the case of benzoic acid and benzamide, the amount of acidic material removed from the ether extract by light petroleum was very little greater than from the extract of normal urine, indicating that there was no appreciable amount (less than 0·5 %) of free phenylacetic acid present. Tulane & Lewis (1933) found that 0·4–3·1 % of a dose of phenylacetic acid was excreted unconjugated.

Phenaceturic acid was readily isolated from the urine after administering phenylacetamide by acidification followed by ether extraction. No unchanged phenylacetamide was detected.

Table 2. *Excretion of 'extra' ether-soluble acid (calculated as phenaceturic acid) after doses of phenylacetic acid and phenylacetamide*

| Substance | Rabbit no. | Dose (g.) | 'Extra' acid excreted in 1st 24 hr. (g.) | 'Extra' acid excreted in 2nd 24 hr. (g.) | 'Extra' acid per g. acid or amide given (g.) 1st 24 hr. | 'Extra' acid per g. acid or amide given (g.) 2nd 24 hr. | Percentage of dose excreted as ether-soluble acid in 1st 24 hr. | Percentage of dose excreted as ether-soluble acid in 2nd 24 hr. |
|---|---|---|---|---|---|---|---|---|
| Phenylacetic acid | 91 | 1·5 | 1·07 | 0·83 | 0·71 | 0·55 | 50 | 39 |
| | | 1·5 | 1·13 | 0·25 | 0·75 | 0·17 | 53 | 12 |
| | 99 | 1·5 | 0·96 | 0·10 | 0·64 | 0·07 | 45 | 5 |
| | | 1·5 | 2·06 | 0·30 | 1·37 | 0·20 | (96) | 14 |
| | | 1·5 | 1·46 | 0·00 | 0·97 | 0·00 | 63[e] | 0 |
| | 116 | 1·5 | 1·17 | 0·54 | 0·78 | 0·36 | 55[f] | 25 |
| | | | | | | Average | 53 | |
| Phenylacetamide | 91 | 1·5 | 1·15 | 0·75 | 0·76 | 0·50 | 54 | 35 |
| | | 1·1 | 0·80 | 0·00 | 0·72 | 0·00 | 51 | 0 |
| | | 1·5 | 1·14 | 0·25 | 0·75 | 0·17 | 53 | 12 |
| | 99 | 1·5 | 1·29 | 0·25 | 0·85 | 0·17 | 60 | 12 |
| | | 1·5 | 1·36 | 0·10 | 0·90 | 0·07 | 63 | 5 |
| | | 1·5 | 1·08 | 0·00 | 0·72 | 0·00 | 51[h] | 0 |
| | 116 | 1·5 | 0·52 | 0·42 | 0·35 | 0·28 | (25)[g] | 20 |
| | | | | | | Average | 55 | |

See footnote to Table 1.

*Ethereal sulphate excretion.* The normal range of daily ethereal sulphate excretion for six rabbits was found to be from 8 to 36 mg. $SO_3$, the average values for individual rabbits ranging from 18 to 24 mg. After the administration of each of the four compounds under investigation, in no instance did the ethereal sulphate excretion increase decisively, for in the cases where a small increase appeared to have taken place, the values were still within the normal range, as is shown in Table 3, which contains typical results.

Table 3. *Ethereal sulphate excretion after administration of acids and amides*

| Rabbit no. | Dose | Excretion of ethereal sulphate in 24 hr. after dose (mg. $SO_3$) | Average normal daily excretion of ethereal sulphate (mg. $SO_3$) | Normal range of daily ethereal sulphate excretion (mg. $SO_3$) |
|---|---|---|---|---|
| 84 | 1·7 g. sodium benzoate | 22 | 26 | 18–36 |
| 116 | 1·5 g. benzamide | 33 | 22 | 11–26 |
| 99 | 1·5 g. benzamide | 22 | 22 | 8–33 |
| 91 | 1·5 g. benzamide | 20 | 18 | 8–33 |
| 91 | 1·5 g. phenylacetic acid | 19 | 18 | 8–33 |
| 106 | 1·5 g. phenylacetamide | 30 | 19 | 11–26 |
| 116 | 1·5 g. phenylacetamide | 14 | 22 | 11–26 |

Even if the slight increases in the cases of the amides were taken to indicate the occurrence of a small amount of oxidation with subsequent conjugation with sulphate, the extent of this would certainly be less than 2 % of the dose given. None of the urines examined gave any coloration with ferric chloride after acid hydrolysis followed by neutralization.

Table 4. *Glucuronic acid conjugation of acids and amides*

| Rabbit no. | Dose | 'Extra' reducing substance excreted during 24 hr. after dose (calc. as glucuronic acid) (mg.) | Percentage of dose conjugated with glucuronic acid in 24 hr. |
|---|---|---|---|
| 84 | 1·7 g. benzoic acid | 197 | 7·1 |
| 99 | 1·7 g. benzoic acid | 459 | 16·6[d] |
| 106 | 1·7 g. benzoic acid | 156 | 5·7 |
| 116 | 1·7 g. benzoic acid | 415 | 15·0[a] |
| | | Average | 10·7 |
| 84 | 1·5 g. benzamide | 103 | 4·2 |
| 99 | 1·5 g. benzamide | 111 | 4·5[c] |
| 106 | 1·5 g. benzamide | 56 | 2·3 |
| 116 | 1·5 g. benzamide | 139 | 5·3[b] |
| | | Average | 4·2 |
| 99 | 1·5 g. phenylacetic acid | 181 | 8·3[e] |
| 106 | 1·5 g. phenylacetic acid | 93 | 4·3 |
| 115 | 1·5 g. phenylacetic acid | 111 | 5·1 |
| 116 | 1·5 g. phenylacetic acid | 66 | 3·0[f] |
| | | Average | 5·2 |
| 99 | 1·5 g. phenylacetamide | 137 | 6·3[h] |
| 106 | 1·5 g. phenylacetamide | 58 | 2·7 |
| 115 | 1·5 g. phenylacetamide | 27 | 1·2 |
| 116 | 1·5 g. phenylacetamide | 123 | 5·6[g] |
| | | Average | 4·0 |

See footnote to Table 1.

*Normal reducing value of rabbit urine.* Determinations of the reducing value of rabbit urine showed that the normal range was, in general, equivalent to 50–200 mg. glucuronic acid in 24 hr., average values for single rabbits ranging from 130 to 177 mg. These values agree with those (140–150 mg.) found by Hanson, Mills & Williams (1944), using a method of estimation based on the naphthoresorcinol colour reaction.

*Reducing values after the administration of acids and amides.* The results obtained are shown in Table 4, which summarizes the excretion of 'extra' reducing substance (expressed as glucuronic acid) and gives the percentage of the doses conjugated with glucuronic acid and excreted in the 24 hr. immediately after dosing. In every case the reducing values during the second 24 hr. were within the normal range.

## DISCUSSION

From the results presented above it appears that, in the rabbit, the metabolism of benzoic acid and benzamide, and of phenylacetic acid and phenylacetamide, is similar in all the aspects studied, since the increases in the urinary excretion of ether-soluble acid, assumed for the purpose of this investigation to consist of the glycine conjugates, and of copper-reducing substances, assumed to be glucuronic acid, are comparable in the case of each acid and amide. The only marked difference is between the increase in glucuronic acid excretion after administering benzoic acid and benzamide, the acid causing a greater increase than the amide (Table 4). This is hardly evident in the case of phenylacetic acid and phenylacetamide. The reason for this difference is not at present clear. Our further experiments upon substituted acids and amides may indicate whether it is confined to the unsubstituted compounds or is more general.

It would seem, then, that the rabbit is able to hydrolyze the amide group to form the corresponding acid, which is then detoxicated. Our results show that, whereas the hydrolysis of benzamide is virtually complete in 24 hr., only about 60 % of phenylacetamide can be accounted for in that time. The similar behaviour of phenylacetic acid suggests that the remaining 40 % of the amide is not excreted unchanged but is detoxicated and eliminated more slowly than benzamide. It is of interest to note that the presence of other groups in the benzene ring affects the stability of the amide group, since we have observed that *m*- and *p*-aminobenzamides are excreted partly as their *N*-acetyl derivatives, with the amide group intact. These compounds are at present under investigation.

Inasmuch as the unsubstituted amides are converted to the acid in the rabbit, it may be argued that the introduction of a hydroxyl group for detoxication purposes is unnecessary. The fact that in our investigation we found no appreciable increase in ethereal sulphate suggests that no oxidation of this type occurs. Baumann & Herter (1877) found, in the dog, a slight increase in ethereal sulphate after benzamide, but also concluded that it was too small for any definite conclusion to be drawn. We have not been able to exclude finally the possibility that a small amount of an ether-type glucuronide may be formed, but if it is formed, the quantity is certainly very small. It appears, indeed, that oxidation followed by conjugation with sulphate or glucuronic acid does not occur to any appreciable extent.

Our findings do not explain the pharmacological difference between the acids and their amides. At the dose levels used the acids are without toxic action, but both amides may have a mild narcotic action, phenylacetamide sometimes causing paralysis. The site of the conversion of an amide to its acid is not yet definitely known. It might be brought about by proteolytic enzymes in the alimentary tract or it might take place after absorption, for example in the liver. In support of the latter possibility Gonnermann (1902) obtained evidence that benzamide could be hydrolyzed by sheep liver and kidney, but not by pepsin or trypsin. A further study of the hydrolysis of amides by enzymes and tissues would, however, be of value, for it might provide a clue to the reason for these differences in action which cannot be explained by the study of the urinary excretion of detoxication products. The simplest explanation of the differences observed is that the narcotic action is a property of the amide itself, and that the process of detoxication, which converts it to the corresponding (non-toxic) acid, is not complete, leaving some unchanged amide to exert its toxic action.

## SUMMARY

1. The metabolism of benzoic acid and benzamide and of phenylacetic acid and phenylacetamide in the rabbit has been studied by the determination of the increase in excretion of ether-soluble acid (calculated as hippuric or phenaceturic acid) and reducing substance (calculated as glucuronic acid) caused by their administration.

2. No appreciable difference was found between the metabolism of the acids and their amides, except that benzoic acid gave rise to a somewhat greater increase in reducing substance excretion than did benzamide.

3. The percentage of a dose of benzoic acid excreted in 24 hr. conjugated with glycine was 77, and with glucuronic acid 11. The corresponding values for benzamide were 76 and 4, respectively.

4. The percentage of a dose of phenylacetic acid excreted in 24 hr. conjugated with glycine was 53, and with glucuronic acid 5. The corresponding values for phenylacetamide were 55 and 4, respectively.

5. No significant increase in the excretion of ethereal sulphate was observed after the administration of any of the four compounds under investigation.

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# Toxic Effects of Oxygen and of Hydrogen Peroxide on Brain Metabolism

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This paper describes the nature of the toxic action of oxygen upon the respiration of minced brain tissue, and gives an account of experiments undertaken to discover how oxygen at 1 atm. pressure inhibits brain respiration. These experiments are of a preliminary nature and the conclusions therefore tentative.*

## RESULTS

### *Rates of respiration of minced rat brain tissue in air or in oxygen*

The respiration of minced rat brain was examined in a Warburg manometric apparatus at 37° in an atmosphere of air or of oxygen. The tissue was minced with a scalpel or scissors, as quickly as possible after removal of the brain from the animal. It was well mixed with four times its weight of 0·9 % (w/v) NaCl solution, and 1 ml. of the suspension was added to a sodium phosphate buffer (pH 7·4 0·02 M)-Locke medium (NaCl 0·13 M; KCl 0·004 M; $CaCl_2$ 0·002 M) in a Warburg manometric vessel. The rates of oxygen uptake were recorded either in the absence of any added substrate or in the presence of 0·027 M-sodium lactate or 0·027 M-sodium pyruvate or 0·05 M-sodium succinate. When glucose was added, its final concentration was 0·01 M.

In Table 1 are typical results showing the rates of respiration of the tissue under the varying conditions. In the absence of added substrate, the rate of respiration of the minced tissue diminishes rapidly with time, falling off more rapidly with oxygen than with air, and the rate of fall is greatest in the third hour. The respiration in the third hour is, with air, about 35 % of that in the first hour, whilst, with oxygen, it is about 20 % of that in the first hour. At the end of the third hour, the tissue is respiring about twice as rapidly in air as in oxygen.

When glucose is present as substrate, the same phenomenon is seen (Exps. 3 and 4, Table 1), the rate of fall of respiration in the third hour with oxygen present being much greater than with air present. The percentage fall in the rate of respiration in one experiment was 33 with air, and 75 with oxygen (Table 1). The brain tissue may be respiring in air at the end of the third hour at even double the rate of that in oxygen, though the initial rates of respiration may be higher in oxygen than in air.

The same phenomenon is exhibited by brain in the presence of sodium lactate (Exps. 5 and 6,

* The work was discontinued early in 1941 to enable the authors to undertake other scientific work, and was reported to the Medical Research Council in the same year in view of the practical importance of toxic effects of oxygen at high pressure. Publication of the results was not allowed at the time. Subsequent to the finding of the facts stated in this paper came the publication of Elliott & Libet (1942) whose results largely confirm those stated here, and the review on oxygen poisoning by Stadie, Riggs & Haugaard (1944). Our results form the starting point of the more exhaustive work of Dr F. Dickens whose papers follow this (Dickens, 1946*a*, *b*).