The study by Pappenheimer & Shaskan (1944) of the effect of iron concentration on the glucose metabolism by *Cl. welchii* strikingly illustrates one function of iron in the growth of this organism. These workers found that the products of glucose breakdown depended upon the iron content of the cells; as the iron content decreased the reaction shifted from a predominantly acetic-butyric acid type with production of large amounts of carbon dioxide and hydrogen, towards a more purely lactic acid type of fermentation with slight gas formation. Under the conditions of their experiments the iron concentration for maximum growth, optimum toxin production and minimum lactic acid production were identical. But the relationship between α-toxin (lecithinase) production and the carbohydrate metabolism was not a direct one, since the shift to a lactic acid type fermentation at low iron concentrations occurred equally well under conditions otherwise unsuitable for α-toxin formation, i.e. in the absence of the toxigenic factor. The toxigenic factor studied in the present work is only one factor which influences toxin formation. The nature of the relation between iron concentration and toxigenic factor in toxin formation, which again may be indirect, remains to be discovered.

SUMMARY

1. Protein-free horse muscle extract contains labile material which enhances α-toxin (lecithinase) formation by *Cl. welchii* type A (strain S 107).

2. Some general properties of the partially purified toxigenic factor have been studied, which suggest that the active component may be of the nature of an amino-sugar.

3. The inorganic iron concentration, in some media, is very important in its effect on α-toxin formation by *Cl. welchii*.

The work recorded in this paper was begun during 1941 as part of a study of toxin formation by bacteria of the gas-gangrene group. Later this was incorporated into a programme of research initiated by a group of workers, eventually organized as the Toxoid team, Anaerobe Sub-Committee of the War Wounds Committee of the Medical Research Council. The work described here lay outside the main programme of the Toxoid team as it eventually developed, and was discontinued when the authors took up other aspects of the problem; it is thus suitable for publication separately. Our sincere thanks are due to Dr J. W. Trevan, F.R.S., Wellcome Physiological Research Laboratories, Beckenham, and to Mr R. L. Grant, Wellcome Chemical Works, Dartford, for supplying us with large quantities of the horse-muscle extract.

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The Metabolism of Sulphapyridine in the Rabbit

By H. G. BRAY, F. C. NEALE and W. V. THORPE, *Department of Physiology, Medical School, University of Birmingham*

(*Received* 18 *February* 1946)

Although the metabolism of sulphapyridine has been the subject of many investigations, much of the work has been concerned only with the isolation and identification of its excretion products or with single aspects of its metabolism. The earlier studies were carried out before it was appreciated that the oxidation of sulphapyridine occurred as a normal metabolic process, but recent investigations, such as those of Gilligan (1945), in which the metabolism of several sulphonamides in man was investigated; of Scudi & Jelinek (1944), which was concerned with the role of urinary excretion products of these compounds in urolithiasis, and of Maher (1944), which deals with the part played by the reticulo-

endothelial system in sulphonamide activity, have emphasized the need for more detailed investigations of all possible aspects of the problem.

Sulphapyridine may be excreted either unchanged, as the free drug or its acetyl derivative, or may be oxidized to a hydroxy derivative which might be excreted free, acetylated, or conjugated with sulphuric or glucuronic acids. The excretion of free and acetylated sulphapyridine have frequently been discussed. The occurrence of oxidation was first shown by Scudi (1940) who isolated a hydroxysulphapyridine from the urine of dogs which had received the drug and this was supported by the observation that the glucuronic acid excretion of rats was increased after the administration of sulphapyridine (Scudi & Robinson, 1941). This work was amplified later (Weber, Lalich & Major, 1943; Scudi, 1944).

We have also obtained from the urine of rabbits dosed with sulphapyridine, a hydroxy derivative which appears to be identical with that obtained from the dog. Although we have not yet been able to establish its constitution finally, we can confirm the conclusion reached by the American workers that the hydroxyl group is attached to the pyridine ring, since on acid hydrolysis it gives sulphanilic acid and not an aminophenolsulphonic acid as would be expected if the hydroxyl group had been introduced into the benzenoid part of the molecule. It is of interest that another *N'*-substituted sulphanilamide of which a hydroxy derivative has been shown to be a metabolic product, viz. sulphaquinoxaline, has the hydroxyl group in the heterocyclic portion of the molecule (Scudi & Silber, 1944).

A feature of almost every study reported in the literature is the comparatively high percentage of ingested sulphapyridine which cannot be accounted for by urinary excretion, when methods of analysis dependent upon the presence of an amino group are used. Thus Long & Feinstone (1938) were able to account for 39–79% in man; Schmidt & Hughes (1939) 60–65%; Stokinger (1939) 15–85%; Ratish, Davidson & Bullowa (1940) 20–83%; and Kinsman, Moore & Harrison (1940) 21–56%. It should be noted that in these studies the dose levels and methods of dosing differed considerably, so that the results are not strictly comparable. Stokinger claims to have accounted for 95–99% in a dog in two experiments, but the dose level was very low (0·167 g./kg.), and it may be significant that the dog does not appear to be able to acetylate aromatic amino-compounds, although it can acetylate amino-acid derivatives such as *p*-bromophenyl-*l*-cysteine (Stekol, 1938). In past investigations in which a considerable proportion of the ingested drug does not appear in the urine it has been concluded that this is due to incomplete absorption. In the present study conditions were arranged so as to achieve the maximum recovery of sulphapyridine. Rabbits, kept under strictly controlled conditions and receiving a constant diet, were given single doses of sulphapyridine at levels of 0·1, 0·25, 0·5 and 1·0 g./kg., and the known excretion products were estimated in the urine (and, in certain cases, in the faeces) passed during subsequent periods of 24 hr. It was not found possible to account for all the drug administered, particularly at the higher dose levels.

INVESTIGATION OF THE EXCRETION OF SULPHAPYRIDINE IN URINE AND FAECES

Methods

Animals. The rabbits used were does weighing 2–3 kg.

Diet. Cabbage (100 g.), bran and oats (25 g. of each, per diem) with water *ad lib.*

Dosing. The drug was given by stomach tube as a suspension in up to 100 ml. warm water. At the dose levels employed it appeared to be without toxic effects. In order to obtain a sufficiently large volume of urine for the analyses on days during which normal urine was collected for analysis, water (100 ml.) was administered by stomach tube. It was established that giving water in this way had no effect on the amounts of metabolites excreted. In some experiments sulphapyridine was injected by way of an ear vein or artery as a 10% solution in dilute NaOH (just sufficient to give complete solution at 37°); the pH was 9·5.

Estimation of ethereal sulphate. Inorganic and total sulphate were estimated by the method of Folin (1905–6). The difference was assumed to be ethereal sulphate.

Estimation of sulphapyridine and its derivatives. These were determined by the method of Bratton & Marshall (1939), using *N*-(1-naphthyl)-ethylenediamine hydrochloride as coupling reagent, and a visual colorimeter of the micro-Duboscq type. Where the solutions were pigmented the results were checked using an instrument of the Burker (comparator) type. The estimation was performed (*a*) on urine as collected, (*b*) on urine after hydrolysis with dilute acid, and (*c*) on urine after 4 hr. continuous extraction with ether. All diazotizable material estimated was expressed as sulphapyridine. Table 1 summarizes the substances estimated by these procedures. It was assumed that the

Table 1. *Estimation of metabolites of sulphapyridine in urine*

Treatment of urine	Compounds estimated
(*a*) Untreated	Unacetylated sulphapyridine, unacetylated hydroxysulphapyridine, including that conjugated with glucuronic acid and sulphate
(*b*) Hydrolysis with dilute acid	Total sulphapyridine and hydroxysulphapyridine, including (*a*) and that originally conjugated with acetic acid
(*c*) 4 hr. Continuous ether extraction	(*a*) Minus sulphapyridine and hydroxysulphapyridine unconjugated with acetic and glucuronic acids and sulphate*

* Some acetyl compounds are extracted with ether, but as the urine is not hydrolyzed before the estimation these are not included in the values obtained.

ethereal sulphate and glucuronide of hydroxysulphapyridine are insoluble in ether. The ether extract was shown by the naphthoresorcinol test not to contain any detectable amount of glucuronic acid. It was also shown that hydroxysulphapyridine is quantitatively estimated by this method giving the same intensity of colour as a molecular equivalent amount of sulphapyridine. Scudi & Robinson (1939) state that alkali is preferable to acid for hydrolysis in the estimation of total sulphapyridine, since acid splits it into 2-aminopyridine and sulphanilic acid, which couples more slowly than sulphapyridine. We found, however, that alkaline hydrolysis of some rabbit urines gives rise to a brown pigment which makes the subsequent matching of colours in a visual colorimeter difficult. Using acid for hydrolysis we have been able to obtain satisfactory results by waiting until no further change in colour took place before attempting to match with the standard. It was not possible to detect the presence of unconjugated hydroxysulphapyridine by the methods employed. The ferric chloride reaction, which was always negative is, in any case, not sufficiently sensitive for the detection of small amounts of hydroxysulphapyridine.

The sulphapyridine content of 24 hr. faeces was determined by extracting them with boiling water or 0·5 N-HCl for 1 hr., making the extract up to a known volume and estimating in the usual way. Hydrolysis of aqueous extracts caused a very small increase in the amount of diazotizable material (less than 5 mg., corresponding to about 10% of the amount present), probably due to acetylated sulphapyridine derived from urine with which the faeces had been in contact in the metabolism cage. Continuous ether extraction of faecal material, either dry or suspended in water, removed less sulphapyridine than did acid extraction. It was shown that the presence of normal faeces in a sulphapyridine solution did not interfere with the estimation of the drug under the conditions described.

Estimation of reducing substances. The method used has been described (Bray, Neale & Thorpe, 1946). In order to estimate the ether-type glucuronide formed by hydroxysulphapyridine it was necessary first to hydrolyze the urine. Complete hydrolysis (i.e. maximum reduction) was obtained by adding $\frac{1}{10}$ vol. of conc. hydrochloric acid and heating in a boiling water-bath for 90 min., cooling, neutralizing with solid Na_2CO_3, making up to a known volume and estimating in the usual way.

RESULTS

Basal excretion levels. For the purpose of this investigation the base-lines from which increases in excretion were calculated were determined by the analysis of a large number of samples of the normal urine of the rabbits being used, both before and during the experimental period. In every case analyses were made on the day immediately preceding the dose and on the day after all the diazotizable material had disappeared from the urine. In general the base-line used was the mean of all these normal values. The following points should be noted:

Diazotizable material. The amount of diazotizable material in normal rabbit urine, both before and after hydrolysis, is negligible, and thus the base-line is zero. In rabbits which have received sulphapyridine the excretion of diazotizable material is essentially complete in 48–72 hr., although the urine passed up to 7 days afterwards may contain, after hydrolysis, 2–3 mg. diazotizable material per day. These small amounts may be due to the process of refection which occurs in the rabbit (cf. Eden, 1945), or, if storage of the drug in the tissues occurs, to a slow release from these stores, but are insignificant compared with the amounts excreted during the first 48 hr. after dosing. The blood also contains small amounts (2–3 mg./100 ml.) up to 7 days after the dose. This may represent sulphapyridine bound to plasma protein (cf. Davis, 1943).

Reducing material. The determination of reducing material (assumed to be glucuronic acid) in this instance is complicated by the fact that the reducing power of normal rabbit urine increases on acid hydrolysis under the conditions used for decomposing the ether-type glucuronide. Separate experiments were made to determine the extent of this increase and it was found to vary from urine to urine, the usual range being from 15–40% of the unhydrolyzed value. The average for all the urines examined was 26·1%, averages for individual rabbits not differing significantly from this. In addition, hydroxysulphapyridine will reduce the Shaffer-Hartmann reagent under our conditions, 3·57 mg. being equivalent to 1 mg. glucose in reducing value. It is, however, possible to calculate the amount of hydroxysulphapyridine which is conjugated with glucuronic acid from the increase in reducing power and the amount of hydroxysulphapyridine conjugated with ethereal sulphate, as follows:

If a be the increase in ethereal sulphate excretion, b the increase in reducing value, expressed as mg. glucose (i.e. the amount in excess of 126·1% of the base-line value), and g the extra glucuronic acid excreted, then the following equations may be deduced:

Total extra reducing material (as glucose) (b)

$$= \frac{\text{Total hydroxysulphapyridine}}{3{\cdot}57} + \frac{g}{1{\cdot}08}$$

(1·08 mg. glucuronic acid ≡ 1 mg. glucose).

Total hydroxysulphapyridine
= that conjugated with sulphate (c)
+ that conjugated with glucuronic acid (d).

Since $c = 3{\cdot}313a$ and $d = 1{\cdot}366g$,

$$g = \frac{b - 0{\cdot}928a}{1{\cdot}307}, \text{ etc.}$$

In this calculation it has been assumed that the amount of unconjugated hydroxysulphapyridine excreted in the urine is negligible. We have obtained evidence that this is the case, since an ether extract of unhydrolyzed urine from a rabbit which had received the drug did not reduce the copper reagent more than a normal urine extract did.

Todd, Dodson, Trainer & McKee (1944) state that sulphanilamide and sulphathiazole interfere with the estimation of glucose by some copper-reducing methods. We found that the presence of sulphapyridine had no effect upon the estimation of glucuronic acid either in water or urine under our conditions.

Distribution of metabolites of sulphapyridine in urine. Table 2 summarizes the quantitative roles of the various metabolites in the urinary excretion of sulphapyridine administered to rabbits at the rate of 0·5–1·0 g./kg.

Acetylation and percentage recovery of sulphapyridine. Tables 2 and 3 show the extent of acetylation which occurs in the rabbit at various dose levels, together with the total amount of the drug excreted. In almost every case excretion was virtually complete in 48 hr. In general, 80–96 % of the total amount excreted appeared in the urine during the first 24 hr.

Examination of faeces. Table 4 summarizes the results obtained. In no case was any diazotizable material found in the faeces after 4 days from the time the dose was administered. The faeces

Table 2. *Distribution of metabolites of sulphapyridine in rabbit urine*

Rabbit	Dose level (g./kg.)	Dose (g.)	Total % of dose in urine[1]	% of dose acetylated[2]	% of dose unacetylated[3]	% of dose unconjugated[4]	% of dose oxidized and conjugated with: Glucuronic acid	% of dose oxidized and conjugated with: SO_3	Total % of dose conjugated through —OH group
84	0·5	1·4	84·1	49·7	34·4	14·4	22·6	0·9	23·5
	0·5	1·4	61·0	24·0	37·0	21·6	25·0	Nil	25·0
91	1·0	2·0	61·0	36·2	24·8	8·5	16·3	4·1	20·4
	1·0	2·1	60·5	34·0	26·5	19·2	17·3	Nil	17·3
	0·5	1·0	70·2	39·0	31·2	6·3	16·7	0·9	17·6
99	1·0	2·1	63·0	40·7	22·3	12·6	18·7	1·1	19·8
	1·0	2·1	33·0	7·3	25·7	12·4	20·7	Nil	20·7
	0·5	1·0	83·5	57·2	26·3	24·4	18·4	Nil	18·4
109	1·0	3·3	43·6	13·6	30·0	15·6	21·0	4·0	25·0

[1] = (*b*), [2] = (*b*)–(*a*), [3] = (*a*), [4] = (*a*)–(*c*), of Table 1.

Table 3. *Effect of dose level on degree of acetylation and percentage of sulphapyridine excreted in rabbit urine*

Rabbit	Approx. dose level (g./kg.)	Dose (g.)	Total % of dose in urine[1]	% of dose acetylated[2]	% of dose unacetylated[3]	% of urinary sulphapyridine acetylated
84	1·0	2·8	36·9	6·3	30·6	17·0
	1·0	2·8	70·2	23·6	46·6	33·6
	0·5	1·4	84·1	49·7	34·0	59·1
	0·5	1·4	61·0	24·0	37·0	39·3
	0·25	0·65	66·8	24·3	42·5	36·4
	0·1	0·26	91·8	51·8	40·0	56·6
106	1·0	2·2	35·6	19·9	15·7	55·9
	1·0	2·2	54·1	33·6	20·5	62·0
	0·5	1·1	62·5	39·2	23·3	62·7
	0·25	0·55	77·3	56·7	20·6	71·9
	0·1	0·22	83·2	64·1	19·1	77·0
	0·1	0·2	90·5	70·0	20·5	77·8
115	1·0	2·0	52·2	5·0	47·2	9·6
	1·0	2·0	56·4	9·7	46·7	17·2
	0·5	1·1	45·5	6·2	39·3	13·6
	0·25	0·5	75·4	22·8	52·6	69·7
	0·1	0·2	95·5	39·0	56·5	40·7
116	1·0	1·7	47·5	32·2	15·3	67·7
	0·5	0·85	61·7	34·8	26·9	56·4
	0·5	0·85	65·4	49·7	12·7	76·0
	0·25	0·45	79·1	62·0	17·1	73·2
	0·1	0·2	71·5	57·5	14·0	80·4
	0·1	0·17	93·9	79·2	14·7	84·7

[1], [2] and [3] as in Table 2.

Table 4. *Sulphapyridine in rabbit faeces*

Rabbit	Dose level (g./kg.)	Dose (g.)	Total % in urine	Total % in faeces in 96 hr.	Total % recovered
84	1·0	2·8	70·2	2·6	72·8
	0·25	0·65	66·8	1·2	68·0
99	1·0	2·1	63·0	2·7	65·7
106	0·1	0·22	83·2	Nil	83·2
	1·0	2·2	54·1	4·6	58·7
109	1·0	3·3	43·6	3·7	47·3
115	0·1	0·2	95·5	Nil	95·5
116	0·5	0·85	65·4	3·0	68·4
	0·25	0·45	79·1	3·7	82·8

Table 5. *Urinary excretion of injected sulphapyridine*

Rabbit	Approx. dose level (g./kg.)	Dose (g.)	Total % in urine	% unacetylated in urine	% acetylated in urine
99	0·5	1·0	89·3	30·6	58·7
106	0·5	1·0	87·2	31·9	55·3
115	0·5	0·9	78·4	63·0	15·4
116	0·5	0·8	81·0	27·0	54·0

examined were all 'day' faeces. No attempt was made to examine 'night' faeces.

Action of intestinal contents on sulphapyridine solutions

Solutions containing known amounts of sulphapyridine (1 : 5000 or 1 : 10,000) were incubated at 37° with some of the contents of the (*a*) ileum, and (*b*) caecum of freshly killed rabbits. With the former no change in the amount of the drug could be shown up to 48 hr., but in several experiments caecal contents appeared to cause a small loss of the drug in 24 hr. A similar loss was observed when the drug was estimated immediately on mixing the solution with the intestinal contents, and this loss did not increase on incubation. This loss, therefore, appears to be due to some sort of adsorption, either physical or by chemical union, rather than to the action of enzymes* or bacteria. Normal ('day') faeces did not have the same effect when ground with water and mixed with a solution of the drug in the cold. Whatever the cause of this loss, it is certainly not great enough to account for more than a very small part of the sulphapyridine untraced in our experiments, particularly those at dose levels of 0·5 and 1·0 g./kg. Even for the smaller of these dose levels the weight of caecal contents required to account for the missing drug would be of the order of 12·5 kg.

Injection experiments. In the hope of distinguishing between losses occurring in the alimentary tract and losses in unknown metabolic processes, experiments were carried out in which the drug was injected intravenously. That the losses mentioned are only pronounced at higher dose levels can be seen from Tables 2 and 3. Unfortunately it is not practicable to inject the greatest doses, for we have confirmed the findings of Marshall & Long (1939) that the highest safe dose level for injection into rabbits is 0·5 g./kg. While such doses have no ill-effects on some rabbits, with others they cause convulsions starting about 5 min. after injection and lasting for 2–3 hr. Local necrosis of the ear was observed in some rabbits.

The results from injection of doses of 0·5 g./kg. are shown in Table 5, which gives the percentages of the doses appearing in the urine and the amount of acetylation which occurs. No diazotizable material was found in the faeces after doses of 0·2 g./kg., but after the higher doses 2–3 mg./day were detected. This amount is very much smaller than that found after giving the same dose by stomach tube. These findings support those of Barber, Dible & Haslewood (1943) who found that the drug is excreted in the bile of rabbits in only very small amounts. The percentage of the dose acetylated was similar to that occurring on feeding by stomach tube. The percentage recovery is definitely greater than that achieved by feeding at the same dose level.

ISOLATION AND PROPERTIES OF HYDROXYSULPHAPYRIDINE

This compound was isolated via its glucuronide by a method based on that of Weber *et al.* (1943).

Urine from rabbits was collected for 48 hr. after they had received doses of sulphapyridine (2 g.), and was continuously

* Digestion of solutions of sulphapyridine with pepsin and pancreatin at their optimum pH's for 21 hr. did not affect their quantitative diazotization and coupling.

extracted with ether for 50 hr. and then acidified with acetic acid. Excess saturated lead acetate solution was added and the precipitate removed at the centrifuge and discarded. The supernatant liquid was made just alkaline with 10% NaOH and the resulting precipitate centrifuged off and suspended in water. An excess of H_2S was passed and the lead sulphide formed filtered off. The filtrate gave intensely positive naphthoresorcinol and diazo reactions. It was concentrated under reduced pressure to $\frac{1}{8}$ vol. and $\frac{1}{10}$ part by weight of solid NaOH added and the solution refluxed for 5 hr. The mixture made just acid to litmus with 2 N-HCl and continuously extracted with ether for 36 hr. After about 30 hr. crystalline material began to separate from the ether extract in the form of large yellow plates. Yield: 2 g. from 20 g. sulphapyridine fed.

Immediately after separation the crystals had m.p. 90–100°, but on standing in air for some days they became opaque and the melting-point rose to 185° (cf. Scudi, 190°; Weber, Lalich & Major, 180°). Recrystallization from water did not raise the melting-point further. The mixed melting-point with sulphapyridine (m.p. 192°) was 155–158°. The compound gave a yellow coloration with acid nitrite, but coupled normally and with ferric chloride it gave a violet coloration which rapidly changed to a red-brown precipitate. (Found: C, 50·01; H, 4·37; S, 11·8; N, 15·8. Calc. for $C_{11}H_{11}O_3N_3S$: C, 49·82; H, 4·15; S, 12·08; N, 15·85%).

Hydrolysis of this compound for 3 hr. with 10% HCl gave a solution from which, after neutralization, a picrate could be precipitated in the form of fine needles by the addition of picric acid. It was recrystallized from water and melted at 218–219°. (Found: Picric acid, 67·9; N, 20·4. *Monopicrate of* $C_5H_6N_2O$ requires picric acid, 67·55; N, 20·65%.) It could be decomposed by suspending in dilute HCl and extracting with ether. The acid aqueous solution was evaporated to dryness and the residue dissolved in the smallest necessary amount of ethanol and from this solution a syrup could be precipitated with ether. This partially crystallized, but it has not yet been possible to isolate material pure enough for analysis. The crude product melted at 34° and gave a purple coloration with ferric chloride, but no diazo reaction. It gave a slight yellow coloration with acid nitrite.

Evaporation of the acid hydrolysate to small bulk gave crystals which melted above 300° and gave a positive diazo reaction but no coloration with ferric chloride. (Found: C, 41·82; H, 4·16; S, 18·04; N, 8·04. Calc. for $C_6H_7O_3NS$: C, 41·62; H, 4·05; S, 18·5; N, 8·09%.) It thus seems reasonable to conclude that the compound isolated from urine is a hydroxysulphapyridine in which the hydroxyl group is attached to the pyridine ring. The compound obtained via its picrate is presumably a hydroxy derivative of 2-aminopyridine. Synthetic studies in this series are difficult and no hydroxyaminopyridines are described in the literature. Experiments are being carried out in an attempt to identify this moiety of the molecule.

It was hoped to obtain a hydroxyaminopyridine as an excretion product of 2-aminopyridine in the rabbit. 2-*N*-acetaminopyridine was administered to 2 kg. rabbits, but it was found to be too toxic to continue, since a dose of 1·5 g. was invariably fatal within a few hr. One rabbit receiving 1 g. survived for 24 hr. The urine passed by these rabbits on diazotizing and coupling with dimethyl-α-naphthylamine gave a faint orange colour, similar to that given by 2-aminopyridine itself. By extraction of the urine with ether a small amount of syrupy material was isolated, which on treatment with acetic anhydride gave a small amount of crystalline material, melting at 152–153°. This substance was not 2-*N*-acetaminopyridine. An attempt to obtain an aminopyridine derivative from the urine by way of the glucuronide (as above) was unsuccessful. The urine as collected gave only a feebly positive naphthoresorcinol test.

DISCUSSION

Perhaps the most interesting of the conclusions which may be drawn from the results presented above is that, as the dose level falls, the percentage of sulphapyridine which can be accounted for in the urine increases, until at 0·1 g./kg. about 86% can be detected. Parallel with this increase in recovery is an increase in the relative amount of acetylation that occurs. At higher dose levels the percentages of both acetylation and the excretion in the urine are rather variable. Much less variation occurs in the amount of unacetylated drug excreted, although there are individual differences between different rabbits, and in every case it is more or less independent of the dose level. Fig. 1 represents the averages for all the rabbits used; the curves for individual rabbits were all of similar shape.

The fact that the main differences observed in the amount and nature of the excretion products of sulphapyridine appear to be due chiefly to variations in the excretion of the acetyl derivative suggests that the normal metabolic processes of the rabbit are not able to deal with the whole of larger doses of the drug, and that the capacity of the acetylation mechanism in particular becomes strained. It should be noted, however, that at higher dose levels the absolute amount of the acetyl compound formed is greater than at the lower levels, although it represents a smaller percentage of the dose administered.

There are, in the main, three ways in which the observed results may arise: (*a*) by failure to absorb all the drug at higher dose levels, (*b*) by processes of

detoxication, or of storing the drug, which, if they occur at all at low dose levels, have a role which is insignificant compared with acetylation, and (*c*) by complete katabolism.

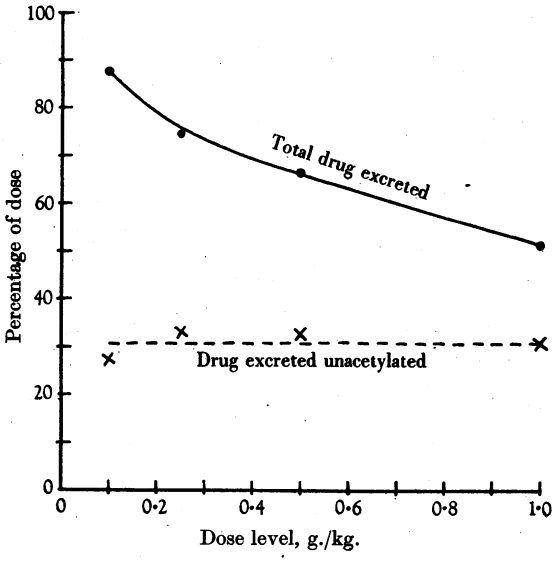

Fig. 1. Effect of dose level on the percentage of sulphapyridine excreted acetylated and unacetylated.

(*a*) It is difficult to reconcile the fact that we found only a small amount of sulphapyridine in the faeces with the suggestion that the loss is due to a failure to absorb the whole dose. An alternative reason for the loss may be that intestinal bacteria (or enzymes) are able to modify the unabsorbed drug by removing the amino group so that it is no longer detectable by diazotizing and coupling. Our experiments with intestinal contents suggest that this does not occur to any appreciable extent. On the other hand, the experiments in which the drug was injected might be interpreted as evidence of incomplete absorption from the alimentary canal. It is, however, to be expected that the response evoked in the rabbit by a sudden increase in the blood concentration of the drug, such as that resulting from injection, might be different from that due to a gradual increase, as when it is absorbed from the alimentary tract. Thus it would seem that close comparisons of the results of experiments of these two types are not possible, and the underlying reasons for our observations may have to be sought elsewhere.

(*b*) There is the possibility that the absence of appreciable amounts of sulphapyridine from the faeces does, in fact, mean that almost complete absorption invariably occurs, in which case the failure to detect all the drug in the urine may be due either to storage in some organ or tissue (the binding by protein of sulphonamide drugs (q.v.) may be of significance here), or to conjugation of the amino group in such a way that mild hydrolysis will not give rise to a group which can be diazotized and coupled. Two such possibilities are (i) methylation, and (ii) introduction of an hydroxyl group in an *ortho*-position to the amino group. The first of these is unlikely since methylation of an aromatic amino group *in vivo* has never been observed, although methylation of the nitrogen atom in pyridine has long been known to occur in some animals, e.g. the dog, but not in the rabbit. The second possibility has, however, been investigated (Thorpe & Williams, 1941; Williams, 1945). Compounds containing an *o*-aminophenolic grouping will, in general, diazotize, but the resulting diazo group reacts with the adjacent hydroxyl group to form a diazo-oxide, which will not couple. Diazo-oxides give yellow solutions, so that compounds containing *o*-aminophenolic groupings yield yellow or orange colorations in solution when treated with acid nitrite. In unhydrolyzed urines it would be expected that such compounds would be found conjugated with glucuronic or sulphuric acids, leaving the amino group free to diazotize and couple in the normal way. We observed that the hydrolyzed urines of rabbits dosed at the higher levels gave similar yellow colorations with acid nitrite, but all the specimens of hydroxysulphapyridine isolated during this investigation also gave yellow colours under the same conditions. Therefore no conclusion can be drawn from these observations as to the occurrence of *ortho*-oxidation in the benzene ring in sulphapyridine. It is difficult to dismiss finally the possibility that the yellow coloration given by hydroxysulphapyridine is due to a small amount of, for example, 2(*p*-amino-*m*-hydroxybenzenesulphonamido)-pyridine, but we consider this unlikely since the intensity of this colour is similar to that of the colour given by pure *p*-amino-*m*-hydroxybenzenesulphonamide and repeated recrystallization does not diminish it. This view is supported by the fact that quantitative estimation of solutions of hydroxysulphapyridine gave results equivalent to 98% of theory. The only alternative method of detoxication of an amino group found in the literature refers to the formation of hydroxylamino compounds (e.g. James, 1940). Claims to have isolated such compounds from urine after the administration of sulphanilamide have been discussed critically by Thorpe, Williams & Shelswell (1941), who concluded that there is not yet sufficient evidence for their formation. It should, however, be mentioned that hydroxylamino compounds, if formed, would diazotize and couple. Actually we were unable to detect any such compounds by either the Pucher & Day (1926) or the Rosenthal & Bauer (1939) reactions.

(c) It has been postulated by Quick (1932) that glucuronide formation may be a preliminary to katabolism, since the feeding or injection of menthol or benzoyl glucuronides results in very little of the parent compounds appearing in the urine. In this connexion it may be significant that in three experiments out of nine at dose levels of 0·5 and 1·0 g./kg. a marked increase (of the order of 150–200 mg. SO_3 in 24 hr.) in inorganic sulphate excretion occurred. That this amount corresponds roughly to the amount of sulphapyridine unaccounted for suggests that it may have arisen from complete oxidation of this part of the dose, but as this increase occurred only occasionally and as no other evidence of the complete destruction of the drug has been obtained so far, it is unwise to draw any such conclusion at this stage.

SUMMARY

1. In the rabbit, both the percentage of a dose of sulphapyridine which can be detected in the urine by the diazo reaction and the amount of acetylation which occurs can be correlated with the dose level at which it is administered.

2. At dose levels of 1·0, 0·5, 0·25 and 0·1 g./kg., the average percentages which can be accounted for are 51·2, 66·7, 74·6 and 87·7 respectively, and the corresponding average percentages of the dose acetylated are 20·2, 33·9, 41·4 and 60·3. The percentage of the dose excreted unacetylated appears to be independent of the dose level, the average percentages being 31·0, 32·8, 33·2 and 27·4.

3. At dose levels of 0·5 and 1·0 g./kg., 17–25 % of sulphapyridine is oxidized and, for the most part, conjugated with glucuronic acid. Sulphate plays little or no part in detoxication under these conditions.

4. The fate of the portion of the dose which cannot be accounted for by urinary excretion, especially at higher dose levels, is discussed. Injection experiments suggest that the loss is due to incomplete absorption, but examination of the faeces does not support this since only very small amounts of the drug (usually less than 4 % of the dose) can be found there.

5. An hydroxy derivative of sulphapyridine has been isolated, via its glucuronide, from the urine of rabbits receiving the drug and its properties are described. It appears to be identical with that obtained by other workers from dogs under similar conditions. There is evidence that the hydroxyl group is attached to the pyridine ring.

The microanalyses were carried out by Drs Weiler and Strauss of Oxford. Part of the cost of this investigation was defrayed by a grant from the Lady Scott-Moncrieff Fund of the University of Birmingham.

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