CCX. THE EFFECT OF ADMINISTRATION OF AN ACETATE ON DETOXICATION AND THERAPEUTIC ACTIVITY OF SULPHANILAMIDE

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ONE of the means by which the animal body protects itself against foreign toxic substances is acetylation as illustrated by the conversion of aniline into acetanilide [Englehart, 1888], and by the acetylation of *m*- and *p*-aminobenzoic acids [Sherwin *et al.* 1926]. Since acetylation also occurs after the administration of sulphanilamide [Marshall *et al.* 1937] it became of interest to enquire whether acetylation would diminish the toxicity of sulphanilamide without affecting its curative value.

The experiments here reported deal with this. Following the lead of other workers [Knoop, 1910; 1911; Hensel, 1915; Sherwin *et al.* 1927; Klein & Harris, 1938], who showed that the acetyl group may be derived from acetates, lactates or pyruvates, sodium acetate was first administered along with sulphanilamide in order to determine whether this would increase the amount of acetyl-sulphanilamide eliminated.

Analytical methods

Sulphanilamide and acetylsulphanilamide were estimated in the mouse urines by the method of Fuller [1937]. For ease of comparison, acetylsulphanilamide has been calculated in terms of sulphanilamide throughout this paper.

The urines were examined for *p*-aminophenol by the indophenol reaction, and the hydroxylamine compound was estimated by the method of Pucher & Day [1926]. *p*-Aminophenol and *p*-hydroxylaminobenzenesulphonamide have been found in the urine of patients treated with sulphanilamide and may be responsible for some of the toxic symptoms; a paper dealing with this subject is in course of preparation.

Table I

	mg./100 ml. free sulphanilamide			mg./100 ml. acetyl- sulphanilamide		
	Actual	Found	% recovery	Actual	Found	% recovery
Unheated urine:						
Decolored	2	2.04	102			
	4	3.92	98			
	8	8.3	104			
Heated urine:						
Decolored	5	4.2	84	5	4.8	96
	10	10.1	101	10	10.6	106
Diluted 25 times	5	2.6	52	5	3.6	72
	10	8.8	88	10	9.2	92

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Preliminary experiments on the recovery of acetylsulphanilamide from mouse urine gave low results after hydrolysing with $2N$ HCl; the results were not improved by dilution but, as Table I shows, decoloring with zinc hydroxide gave better values.

The actual method adopted was to add 2 ml. of 10% $ZnSO_4$ to each 5 ml. heated urine, adjust to pH 7-8 by addition of 10% ammonia and dilute the whole to 10 ml. or other suitable volume, filter and take aliquots of the filtrate for the estimations.

Effect of acetate on production of acetylsulphanilamide

Two groups of 10 mice were used in this experiment which was done to ascertain the effect, if any, that simultaneous dosing with sodium acetate (crystalline) would have on the production of acetylsulphanilamide.

Group A was given 10 mg. sulphanilamide per mouse orally on each of 4 days; then after 3 days with no treatment these mice were each given 10 mg. sodium acetate and 10 mg. sulphanilamide for a further period of 4 days. This was followed by 3 days' rest, at the end of which the mice were killed. During these periods the urine was collected for analysis.

Group B received 10 mg. sulphanilamide and 10 mg. sodium acetate per mouse for the first period of 4 days; 3 days' rest followed, dosage being then continued with 10 mg. sulphanilamide for 4 days and 3 days' rest before killing. As before the urines were collected and analysed. The detailed results are shown in Table II.

Table II

		Group A						Group B			
Period	Dose	Hydroxyl- amine com- pound mg.	Sulphanil- amide		Ratio A/F	Dose	Hydroxyl- amine com- pound mg.	Sulphanil- amide		Ratio A/F	
			Free mg.	Acetyl mg.				Free mg.	Acetyl mg.		
1st 4 days	Sulph.	6.7	27.4	15.6	0.6	Sulph. + acetate	1.7	24.8	27.2	1.1	
2nd 3 days	None	2.3	20.0	13.4	0.7	None	0.0	10.0	5.0	0.5	
3rd 4 days	Sulph. + acetate	2.7	23.5	39.5	1.8	Sulph.	2.0	15.8	3.4	0.2	
4th 3 days	None	0.8	17.2	9.5	0.6	None	0.9	11.0	5.0	0.5	

Table III

Table 111												
Group A							Group B					
Period	Dose	Amino-phenol	Hydroxyl-amine compound mg.	Sulphanil-amide			Dose	Amino-phenol	Hydroxyl-amine compound mg.	Sulphanil-amide		
				Free mg./100 ml.	Acetyl mg./100 ml.	Ratio A/F				Free mg./100 ml.	Acetyl mg./100 ml.	Ratio A/F
1st 4 days	Sulph.	+	2.8	50.0	25.3	0.5	Sulph. + acetate	-	2.5	35.5	21.4	0.7
2nd 8 days	None	++	8.3	10.0	5.2	0.5	None	-	6.8	14.0	11.7	0.8
3rd 4 days	Sulph. + acetate	-	1.8	102.0	76.0	0.7	Sulph.	-	1.7	125.0	25.5	0.2
4th 8 days	None	+	7.5	20.0	8.0	0.4	None	+	3.3	15.1	2.5	0.2

No *p*-aminophenol was detected in any of the urine specimens of Table II although the hydroxylamine compound was present. Examination of human urine has shown that the oxidation products of sulphanilamide are excreted for

some considerable time after cessation of therapy and this probably accounts for the slightly increased excretion of the hydroxylamine compound in the third period with group A which was then receiving acetate in addition to sulphanilamide.

The above cross-over experiment was repeated with two more groups of mice; this time each group containing 8 mice. The amount of acetate was halved but was still more than the theoretical amount necessary entirely to acetylate the sulphanilamide; also the period of rest between doses was increased to 8 days. Table III shows these results.

Tables II and III are summarized in Table IV which shows that administration of acetate increases the amount of acetyl derivative formed during its period of administration and in addition may have some effect in lessening the oxidation products, the improvement appearing to depend on the amount of acetate given especially during subsequent resting periods when there is no difference between the total hydroxylamine outputs.

Table IV

Treatment ...	Sulphanilamide mg. excreted			Sulphanilamide + acetate mg. excreted		
	Hydroxyl- amine compound	Free	Acetyl	Hydroxyl- amine compound	Free	Acetyl
Periods of administration:						
4 days	6.7	27.4	15.6	1.7	24.8	27.2
4 days	2.0	15.8	3.4	2.7	23.5	39.5
4 days	2.8	50.0	25.3	2.5	35.5	21.4
4 days	1.7	125.0	25.5	1.8	102.0	76.0
Totals	13.2	218.2	69.8	8.7	185.8	164.1
Ratio A/F			0.31			0.88
Periods of rest:						
3 days	2.3	20.0	13.4	0.0	10.0	5.0
3 days	0.9	11.0	5.0	0.8	17.2	9.5
8 days	8.3	10.0	5.2	6.8	14.0	11.7
8 days	3.3	15.1	2.5	7.5	20.0	3.0
Totals	14.8	56.1	26.1	15.1	61.2	29.2
Ratio A/F			0.47			0.48

Effect of acetate on antistreptococcal activity

The protective effects of sulphanilamide and sulphanilamide with acetate were compared by infecting groups of mice with lethal doses of group A haemolytic streptococcus, reserving one group of mice as controls and giving five small doses (2.5 mg.) of the drug to each of two other groups of mice at intervals over 48 hr., finally calculating the average life of each series by Whitby's method [1937]. The results of five separate experiments are grouped in Table V.

Table V

Strain	Number of mice in each group	Average life of mice in days		
		Controls	Sulphanilamide	Sulphanilamide + acetate
L 193	8	3.8	10.5	13.0
Richards	8	2.0	11.0	11.0
Richards	8	1.8	12.3	12.0
Richards	6	1.8	6.7	4.1
Richards	5	1.7	12.5	12.0
Average		2.2	10.6	10.4

It appears that acetate does not materially reduce the protective power of sulphanilamide and hence its administration may be advantageous if it reduces acute or chronic toxicity.

The effect of acetate on toxicity

Acute toxicity. The acute toxic effects were produced by giving 100 mg. sulphanilamide orally to each mouse in two groups of 5 mice. In addition, in one of these groups each mouse received 100 mg. sodium acetate.

After 2 hr. the mice receiving sulphanilamide were all distressed and three of them showed the spasticity, inco-ordination and hyperventilation described by Hoare [1939]. At the end of 5 hr. a second dose of 50 mg. sulphanilamide was given; this was very shortly followed by a repetition of the previous symptoms and death of 1 mouse within 24 hr., the remainder recovering with time.

The second group of mice dosed with acetate was also given another dose of 50 mg. sulphanilamide and 50 mg. acetate; these mice showed no symptoms of distress throughout the experiment.

A control experiment giving sodium acetate alone showed no ill effects in any of the 5 mice of this group.

The previous observations were confirmed by repeating the experiment with two groups, each of 6 mice, using the same procedure as before. After 2 hr. all the mice which had received sulphanilamide alone were completely prostrate and spastic, breathing deeply. Two died at 26 and 27 hr. respectively; the other four gradually recovered.

The mice which had received acetate as well as sulphanilamide presented a very striking contrast. Apart from some deep breathing they appeared in good condition after 2 hr. Later one became prostrate and died, the others recovering.

Chronic toxic effect. On account of the alkalosis produced by sodium acetate, it was thought that ammonium acetate might be preferable, but a preliminary experiment with this salt, both alone and with sulphanilamide, showed that more rapidly fatal results were obtained, probably owing to the known toxic effects of the ammonium ion.

Six mice were each given 25 mg. of sulphanilamide twice daily, the dose being increased by 5 and 10 mg. increments until a total dosage of 50 mg. was reached. When the total given was 220 mg. these mice became slow in their movements and after a further 50 mg. lack of co-ordination and hyperventilation were observed, but all mice recovered after a period of 20 hr. from the last dose.

The second group of mice received doses of sodium acetate in the same amount as the sulphanilamide and increasing from 25 to 50 mg. When 220 mg. of sulphanilamide had been given two of these mice showed nervous symptoms and hyperventilation. A further dose of 50 mg. was then given, and another two mice displayed nervous symptoms and over-breathing, one dying 20 hr. after the last dose; the others recovering with time.

A control experiment with doses of sodium acetate graduated in the same manner from 25 to 50 mg. showed no ill effects after a total of 300 mg. had been reached, although as would be expected the urine had a high pH (8.2).

To confirm these results another experiment was performed in which the actual food consumption was determined by weighing the food and also each mouse daily throughout the experiment. As before, two groups of mice were used, one group being fed with 50 mg. sulphanilamide per mouse daily whilst the other group received 50 mg. sulphanilamide and 25 mg. sodium acetate daily. The experiment continued for 17 days before the mice were killed and

during this time the body weight changes and food consumption of each group were almost exactly parallel as will be seen by reference to Fig. 1.

After killing, leucocyte and differential counts were performed and no significant difference was found between the two groups.

Fig. 1.

Administration of acetylsulphanilamide

Curative effect. The slight antistreptococcal activity mentioned by Buttle *et al.* [1936] and by Tréfouel *et al.* [1937] was confirmed on batches of 6 mice administering 18 mg. of acetylsulphanilamide (equivalent to 15 mg. sulphanilamide) in five equal doses to infected mice over 48 hr.; 15 mg. sulphanilamide were also given in similar doses to another batch of infected mice. The average life of the infected controls was 1.8 days (0 survivors for 14 days), that of the acetyl-treated mice was 2.8 days (0 survivors for 14 days) and of the sulphanilamide-treated mice 6.7 days (2 survivors for 14 days).

Acute toxic effect. Marshall *et al.* [1938], giving doses of 2 g. per kg. (i.e. 40 mg. per 20 g. mouse) to a group of 20 mice, reported 9 deaths (45%); with a similar dose of sulphanilamide to a batch of 28 mice, 2 deaths (7.1%) resulted. The deaths all occurred within 4 days.

Using these same doses with a group of 16 mice, 7 deaths (44%) occurred in the same time, thus confirming the results of Marshall. No deaths resulted from treatment of a group of mice with the same dose of sulphanilamide.

When acetate was given along with the acetylsulphanilamide to 8 mice, there was no significant difference in the results as 4 deaths occurred, a mortality of 50%. In an attempt to avoid the deaths occurring on the 3rd and 4th days after acetyl dosage, the acetate was given in doses of 40 mg. each of the first three successive days, without affecting the result.

DISCUSSION

The observation that increased acetylation of sulphanilamide occurred in mice when that drug was administered along with sodium acetate, and the remarkable effect of such simultaneous administration in reducing the acute toxicity of the sulphanilamide suggest at first sight that acetylation is a de-

toxicating mechanism. This interpretation is, however, difficult to reconcile with the observation of Marshall and his colleagues (confirmed by our own experience) that acetylsulphanilamide when administered as such to mice, is definitely more toxic than the parent sulphanilamide.

In face of this apparent discrepancy it is well to bear in mind that the injurious effects of a drug are not always attributable directly to its toxic affinity for certain tissue cells; they may be due rather to the sudden withdrawal of some body constituent in an attempt to detoxicate the drug by synthesis, or to quite different processes such as a profound physical or chemical alteration of the body fluids or again to blockage of an organ such as the kidney.

In the case under consideration here it seems possible that sulphanilamide kills chiefly by sudden withdrawal from the body of acetyl-precursors. The administration of acetylsulphanilamide as such would not make this sudden demand but may well be injurious in other ways.

The co-administration of acetates or acetate precursors and sulphanilamide to human subjects may be important since it should spare the liver and also reduce the amount of the oxidation products of sulphanilamide which produce various toxic reactions in the human. While this has not yet been attempted, it has been found that the dosing with citrates along with sulphanilamide does have some effect in increasing the amount of acetyl derivative formed.

More work is still to be done on this problem and experiments are in progress which may throw more light on the subject, including a histological examination of the tissues of the animals treated by the methods mentioned above.

SUMMARY

Mice receiving sodium acetate along with sulphanilamide, excreted more acetylsulphanilamide than mice receiving the same dosage of sulphanilamide. Such combined treatment did not diminish the curative value of the drug but was effective in modifying the acute toxic symptoms resulting from a single large dose of sulphanilamide. When smaller doses of sulphanilamide were given over a longer period, acetate did not appreciably affect the results.

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REFERENCES

- Buttle, Gray & Stephenson (1936). *Lancet*, **1**, 1286.
Englehart (1888). A contribution to the toxicology of aniline. Franz Diss., Dorpat.
Fuller (1937). *Lancet*, **1**, 194.
Hensel (1915). *Hoppe-Seyl. Z.* **93**, 401.
Hoare (1939). *Lancet*, **1**, 76.
Klein & Harris (1938). *J. biol. Chem.* **124**, 613.
Knoop (1910). *Hoppe-Seyl. Z.* **67**, 489.
— & Kertess (1911). *Hoppe-Seyl. Z.* **71**, 252.
Marshall, Emerson & Cutting (1937). *Science*, **85**, 202.
— — — (1938). *J. Amer. med. Ass.* **110**, 252.
Pucher & Day (1926). *J. Amer. chem. Soc.* **48**, 672.
Sherwin, Muenzen & Cerecedo (1926). *J. biol. Chem.* **67**, 469.
— Harrow & Power (1927). *Proc. Soc. exp. Biol., N.Y.*, **24**, 422.
Tréfouel, Tréfouel (Mme), Nitti & Bovet (1937). *Ann. Inst. Pasteur*, **58**, 30.
Whitby (1937). *Lancet*, **1**, 1517.