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

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In a previous paper [James, 1939] the question of the exact manner by which sulphanilamide and acetylsulphanilamide exerted their toxic action was raised. It was suggested that the acetyl derivative had a direct specific toxic action whilst sulphanilamide might kill by a sudden withdrawal of acetate precursors from the body. The following experiments were undertaken to throw further light on the problem. The work was extended to cover sulphapyridine.

Fuller [1937] was the first to suggest the presence of a conjugated sulphanilamide in urine and Marshall *et al.* [1937] independently isolated this substance from human and rabbit urine. They obtained chemical constants for it and mentioned the previous work of Fuller. In further experiments Marshall *et al.* [1938] demonstrated its increased toxicity as compared with sulphanilamide.

Southworth [1937] was the first to observe a fall in the CO₂ combining power of the blood of patients receiving sulphanilamide; Hartmann *et al.* [1938] therefore suggested the administration of sodium lactate and Lucas & Mitchell [1939] NaHCO₃ to counteract this fall.

Lactate and bicarbonate would both raise the CO₂ capacity but the lactate has the advantage of being an acetyl precursor. During this fall in CO₂ capacity the production of an alkaline urine has been noted [Strauss & Southworth, 1938; Beckmann, 1938]; this is of interest as sulphanilamide is more bactericidal in an alkaline urine than in an acid one [Helmholz & Osterberg, 1937], acetylsulphanilamide being inactive at any reaction.

A fall of CO₂ capacity was also noted in man and dogs by Ocklerblad & Carlson [1939] when giving acetylsulphanilamide. So far these or similar changes have not been reported when sulphanilamidopyridine has been given, but Antopol & Robinson [1939] observed uroliths of acetylsulphanilamidopyridine in kidneys of rats, rabbits and monkeys which had received the base; Gross *et al.* [1939] analysed actual concretions from rat's kidneys and found 64% of the acetylated compound and suggested the possibility of the development of similar calculi in human beings receiving treatment.

Biochemical experiments

Each mouse in 6 groups of 10 mice was given 10 mg. doses of the selected drug on 3 successive days, whilst 3 mice were killed on the 2nd and 3rd days and survivors on the 7th day. There were no fatalities due to the drugs. A group of 10 normal mice was kept under the same conditions and killed at the same time for purposes of comparison. The animals were killed by severance of the

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spinal cord in order that the blood composition should not be changed. Heart blood was then taken under paraffin, and estimations of glucose, O_2 and CO_2 capacities and free and total sulphanilamide (or sulphapyridine) were made. The results are summarized in Table 1. Glucose was estimated by the Folin-Wu colorimetric method.

Table 1. *Blood results in relation to drug concentration (acetylated drug calculated as free for ease of comparison)*

Drug	Day	Drug conc. mg./100 ml.		Glucose mg./100 ml.	O_2 capacity vol.	CO_2 capacity vol.
		Free	Total			
Nil	1	—	0.0	85	16.7	57
	3	—	0.0	82	16.4	55
	7	—	0.0	79	16.5	58
Sulphanilamide	2	18.0	32.5	70	16.5	50
	3	31.0	56.0	63	16.8	45
	7	2.0	3.6	83	16.1	52
Sulphanilamide + acetate	2	15.0	34.5	81	16.3	58
	3	29.0	67.0	80	16.3	63
	7	3.0	6.9	84	16.6	55
Acetylsulphanilamide	2	1.3	6.5	83	16.7	51
	3	2.7	10.8	79	16.3	45
	7	0.2	3.2	78	16.1	48
Sulphapyridine	2	11.0	16.5	75	16.9	52
	3	18.0	27.0	67	16.6	47
	7	8.7	13.0	80	16.7	54
Sulphapyridine + acetate	2	13.3	28.3	82	16.5	56
	3	21.0	46.3	80	16.4	57
	7	10.6	23.3	85	16.6	54
Acetylsulphapyridine	2	1.0	5.1	84	16.7	53
	3	2.1	9.8	79	16.8	50
	7	0.5	3.7	80	16.5	51

It will be seen that the O_2 capacity has not been affected by the drugs but that glucose and CO_2 have varied slightly, both falling in the absence of acetate and rising as the drug is excreted.

Sulphapyridine affects the blood picture in the same way as sulphanilamide does; acetate administration nullifies the depressant effect of both drugs.

The acetyl derivatives of the two drugs behave in a similar manner to each other and, whilst there is a slight fall in blood sugar, there is a marked fall of CO_2 capacity. The simultaneous administration of an acetate does not affect the toxicity of the acetylsulphanilamide [James, 1939] and so no attempt was made to examine the blood picture after the administration of acetates and acetylated drugs.

These results confirm the supposition first made that sulphanilamide kills by sudden withdrawal of acetate precursors whilst the acetyl compound exerts a specific effect. That this is not due to its lower solubility leading to blockage of the kidney tubules, such as occurs with acetylsulphapyridine, is shown by the histological results.

Histological results

The livers, spleens and kidneys of mice which had died as a result of acute toxic experiments with sulphanilamide, sulphanilamide and acetate and acetylsulphanilamide were examined histologically. No structural damage to the tissues was observed. This was confirmed by Dr R. M. Fry, Pathologist of these Laboratories.

DISCUSSION

The use of acetate when administering drugs of the sulphanilamide class offers advantages in modifying the acute toxicity of sulphanilamide [James, 1939] and these experiments show that it acts by preventing the metabolism of acetate precursors. Other substances, such as lactate and bicarbonate, have been suggested to prevent the fall in CO_2 capacity. Acetate has this effect as well as providing acetyl radical directly; in addition an alkaline urine is produced in which sulphanilamide is known to be most bactericidal.

It is known that the acetyl compound is more toxic in mice, but as yet there is no evidence of its toxicity in man, although the acetyl derivative takes a considerable time to eliminate and some individuals may acetylate an extra large quantity.

The fall in glucose can be explained by glucose being an acetate precursor; this hypothesis is strengthened by the fact that when acetate is given this fall is prevented.

SUMMARY

Both sulphanilamide and sulphydrylpyridine have been found to be toxic due to withdrawal of acetate precursors and lowering of the CO_2 capacity of the blood. Acetate given at the same time rectifies this defect.

Acetylsulphanilamide evidently exerts a specific toxic effect although it must also have some effect on the respiratory centre on account of the fall of CO_2 .

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