OBSERVATIONS ON THE EFFECT OF SULPHUR DIOXIDE IN BLACKCURRANT SYRUP ON THE DEVELOPMENT OF ANEURINE DEFICIENCY IN RATS

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Sulphur dioxide, 350 p.p.m., administered orally in blackcurrant syrup, once daily, in a dose of 0·5 ml./150 g. body weight, failed to influence the rate of growth of aneurine deficient rats.

Two facts concerning the toxicity of sulphite are already well known. Firstly, clear evidence has been presented that sulphite treatment of raisins[1], potatoes and cabbage[2] greatly reduces their aneurine content. Secondly, Williams, Waterman, Keresztesy and Buchman[3] demonstrated that the products of interaction between sulphite ions and aneurine molecules had no vitamin B_1 activity. Further evidence of the destruction of the aneurine in a solid diet by sulphite mixed with that diet was provided by Fitzhugh, Knudsen and Nelson[4]. These workers fed young rats with a diet which contained insufficient aneurine to maintain normal growth, and found that the addition of 0·05 per cent of sodium bisulphite, 307 p.p.m. as SO_2, further decreased their growth.

By contrast, no investigation of the effect of the oral administration of sulphite in a fluid medium on the development of aneurine deficiency in animals has been reported. Such an investigation appeared necessary because of the widespread use of sulphur dioxide in a concentration of 350 p.p.m. for the preservation of fruit juices. The experiments reported below constitute a preliminary examination of this problem. A blackcurrant syrup, to which 350 p.p.m. of sulphur dioxide had been added, has been administered orally, once daily to young rats which were fed a solid diet partially deficient in aneurine. The effect of this treatment on the rate of development of aneurine deficiency has been observed.

METHODS

Hooded, inbred, female rats, weighing 85 to 95 g., were divided by weight into three comparable groups. They occupied individual cages with raised wide-meshed wire floors, thus ensuring that their droppings passed out of reach without delay. The atmosphere was well ventillated and thermostatically maintained within a temperature range of 65 to 70° F. Water and the prescribed diets were continually within reach of the animals, which were weighed twice a week.

Experimental diets were derived from a basic diet called D1 which consisted of dextrinised starch, 79 parts, casein, 19 parts, and Steinbock's salt mixture, 4 parts. The basic diet D1 was autoclaved to provide the experimental diets D2 to D4. Diet D2 was autoclaved for 2 hours, D3 for two periods of 2 hours, and D4 for 8 hours. Brewers' yeast 25 parts,

autoclaved for 6 hours, was added to these diets shown in Table I. In addition to the prescribed diet each rat received 0·5 ml. of cod-liver oil weekly by pipette.

The syrups were given by mouth in the early evening shortly before the rats became active and started eating. The control syrup contained sucrose 33 g., glucose 22 g., and fructose 16·5 g./100 ml. of water brought to pH 3·5 with N hydrochloric acid. It contained no sulphur dioxide. The fruit syrup was an extract of blackcurrant, sweetened with sucrose, and to which 350 p.p.m. of SO_2 were added. It had a pH of 3·5 and a composition as follows, sucrose 33·2 g., glucose 21·9 g., fructose 16·6 g., potassium 166 mg., sodium 1·4 mg., ascorbic acid 71 mg., and citric acid 1·7 g./100 ml. Syrups were administered by stomach tube, once a day, in a dose of 0·5 ml./150 g. rat.

Results

The results are summarised in Table I. In the first experiment parallel growth took place in the three groups of rats until the end of the sixth week. Growth continued in control group A throughout the seventh and eighth weeks, but was greatly reduced in groups B and C during the seventh week, and was arrested in these two groups during the eighth week. The rats of groups B and C developed anorexia, muscle weakness, and bradycardia during the eighth week. These symptoms clearly indicated aneurine deficiency in Diet D2. There was, moreover, significant difference in the eighth week between the mean weights of the control animals (group A) and the syrup treated animals fed the reduced aneurine diet (groups B and C); t calculated was 2·49, $P = < 0·05$. Since the rats

TABLE I

THE EFFECT OF A BLACKCURRANT SYRUP, CONTAINING SO_2 350 P.P.M., ADMINISTERED ORALLY, ONCE DAILY, IN A DOSAGE OF 0·5 ML./150 G. RAT, ON THE GROWTH OF RATS MAINTAINED ON DIETS DEFICIENT IN, BUT NOT FREE OF, ANEURINE

Expt.	Group	No. Rats	Mean weights in g. ± S.D. Intervals from onset of special diet and treatment					Diet	Treatment
			End of 4th week	End of 5th week	End of 6th week	End of 7th week	End of 8th week		
I	A	6	135 ± 3·8	146 ± 4·0	153 ± 6·1	162 ± 5·7	168 ± 5·8	D1+yeast	Nil
	B	6	138 ± 5·2	150 ± 5·9	156 ± 5·6	158 ± 5·9	156 ± 6·3	D2+yeast	Simple syrup
	C	5	134 ± 4·5	142 ± 4·5	151 ± 5·1	154 ± 6·2	154 ± 5·7	D2+yeast	SO_2 syrup
			End of 4 weeks	End of 5 weeks	End of 6 weeks	End of 7 weeks	End of 8 weeks		
II	A	5	125 ± 5·2	134 ± 5·4	145 ± 5·6	155 ± 5·8	163 ± 5·5	D1+yeast	Nil
	B	5	133 ± 6·2	143 ± 6·4	150 ± 6·3	155 ± 6·6	148 ± 6·0	D3+yeast	Simple syrup
	C	5	130 ± 5·0	142 ± 5·2	153 ± 5·5	156 ± 5·6	151 ± 5·4	D3+yeast	SO_2 syrup
			End of 3 weeks	End of 4 weeks	End of 5 weeks	End of 6 weeks	End of 7 weeks		
III	A	6	121 ± 5·0	132 ± 5·4	137 ± 5·4	141 ± 6·2	143 ± 6·5	D2 no yeast	Nil
	B	6	123 ± 7·2	130 ± 6·8	132 ± 7·0	134 ± 7·0	132 ± 6·9	D2 no yeast	Simple syrup
	C	6	129 ± 6·4	137 ± 6·5	141 ± 6·8	144 ± 6·8	144 ± 6·6	D2 no yeast	SO_2 syrup
			End of 4 weeks	End of 5 weeks	End of 6 weeks				
IV	A	6	125 ± 4·8	133 ± 5·1	142 ± 5·3			D1+yeast	Simple syrup
	B	6	126 ± 5·2	129 ± 6·5	126 ± 6·1			D4+yeast	Simple syrup

in groups B and C did not differ in their weight gains during the experiment, or in the severity of their symptoms during the eighth week, no toxicity could be ascribed to the fruit syrup containing 350 p.p.m. SO_2 administered to group C.

The diet fed to groups B and C in the second experiment had been increased in aneurine deficiency by further autoclaveing. Rats receiving this diet D3 showed reduced growth in the seventh week, lost weight and developed anorexia, muscle weakness, and bradycardia in the eighth week. Control rats fed diet D1 grew throughout the experiment. At the end of the experiment there was a significant difference between the mean weight per rat in Group A, and the corresponding mean weight for groups C and B; the value of t calculated was 2·61; $P = < 0{\cdot}05$. Again no deleterious effects from the sulphite in the blackcurrant syrup appeared; groups B and C grew, then failed to grow, in parallel.

The third experiment differed from those preceding in two respects: no autoclaved yeast was added to the diet, and all rats received diet D2. Normal growth was maintained in each of the three groups until the end of the fourth week of the experiment, but growth decreased in the fifth week and had ceased by the end of the seventh. Autoclaved yeast was added to the diet for the first three days of the eighth week; a mean weight increase of 2·2 g. $\pm$ 0·8 g. (S.D.) was observed on the 4th day. Autoclaved yeast was replaced by natural brewers' yeast on the 4th day for a period of three days. This produced a further mean weight increase of 6·9 $\pm$ 1·2 g. (S.D.) on the 7th day of yeast treatment. It may be concluded that a deficiency both of aneurine and of the B2 group vitamins had been induced in this experiment. Daily oral treatment with either simple syrup, or blackcurrant syrup containing 350 p.p.m. of SO_2 failed to influence the course of this deficiency.

In the fourth experiment the growth of rats (group A) receiving control diet D1 was compared with that of rats (group B) fed diet D4 which had been autoclaved for eight hours. Autoclaved yeast was added to both D1 and D4, and both groups of rats were treated with the control syrup. Growth proceeded normally in group A throughout the experiment. Failure of growth was observed in group B during the fifth week of the experiment, and the majority of rats in the group lost weight during the sixth week. A significant difference between the mean weights of animals in groups A and B became demonstrable in the sixth week; the value of t calculated was 4·55, $P = < 0{\cdot}01$. The experiment was planned to last for eight weeks, and was continued for this period. One animal in group B died in the seventh week; two further animals in this group died in the eighth. All animals in group B developed anorexia, extreme muscle weakness, and bradycardia, indicative of aneurine deficiency.

Discussion

Rats fed a basic diet which had been autoclaved for 8 hours in experiment IV showed more rapid failure of growth and more severe symptoms of aneurine deficiency than did rats which received the basic diet autoclaved for 2 and 4 hours in experiments I and II. It may therefore be

concluded that the diets used in experiments I, II, and III contained aneurine, but in amounts insufficient to supply the needs of the animals.

Once daily oral administration of a fruit syrup containing 350 p.p.m. of sulphur dioxide in a dose of 0·5 ml./150 g. rat proved no more toxic than a simple control syrup which contained no sulphur dioxide.

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REFERENCES

1. Morgen, *Amer. J. Public Health*, 1935, **25**, 328.
2. Paveck, *Industr. Engng Chem.*, 1946, **38**, 853.
3. Williams, Waterman, Keresztesy and Buchman, *J. Amer. chem. Soc.*, 1935, **57**, 536.
4. Fitzhugh, Knudsen and Nelson, *J. Pharmacol.*, 1946, **86**, 37.