SOME ASPECTS OF THE METABOLISM OF SULFUR DIOXIDE IN ANIMALS AND MAN

by

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INTRODUCTION

Soon after the entry of the United States into World War II it became evident that the problem of supplying our troops with adequate amounts of edible foodstuffs under adverse conditions of temperature and humidity would necessitate, particularly in the case of dehydrated fruits and vegetables, that the then existing commercial practices as to the use of preservatives in the preparation of these foodstuffs would have to be modified.

The preservative ordinarily used in this case is sulfur dioxide, applied either as a solution in the blanching process, or as a gas in the drying rooms.

Early experiments conducted by the Quartermaster Department of the United States Army had demonstrated that these foodstuffs could be adequately protected by the use of amounts of sulfur dioxide in the finished products equal to about twice the then prevailing commercial standards.

Since the use of artificial preservatives in foodstuffs is under strict legal control as a measure of protection of the public health and since in this case it was essential that the relative amount of preservative be greatly increased, it became necessary to determine whether this increase would

have any adverse effect on those persons consuming foodstuffs containing it.

At the request of the Committee on Medical Research of the National Research Council the present study was undertaken, the purpose being to determine any physiological effects of high levels of sulfur dioxide in foodstuffs.*

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HISTORICAL

The use of sulfur dioxide in the preservation of dried fruits is at least a century old. Late in the nineteenth century there was a tendency to increase the amount of this preservative in dried fruits because of the improved appearance of fruits so treated. It was also possible to raise the residual water content of the fruit without subsequent spoilage if the sulfur dioxide content was increased.

This tendency came to the attention of public health authorities in both Europe and America with the result that there appeared two studies of the pharmacological action of the compounds of sulfur dioxide, one in Europe, that of Rost and Franz (1) and the other in America, that of Wiley (2). These were both published in the first decade of this century. Since that time no publications relating to this subject have appeared in the literature. There have been, due to the widespread use of liquid sulfur dioxide in refrigeration, numerous reports as to the effects of gaseous sulfur dioxide upon animals and humans. These are not of interest in connection with this study.

In the studies mentioned above, the source of sulfur dioxide in the case of Rost and Franz was artificially prepared compounds of sulfur dioxide with sugars or sodium bisulfite, and in the case of Wiley, sodium bisulfite only. Both groups used mainly sodium bisulfite in their feeding experiments. The German

workers used from 0.1 gram to 2.5 grams SO_2 equivalent daily per experimental subject. Their work extended over a period of ten years, the last two years being devoted to the study of the effects upon twenty human volunteers. Various amounts of SO_2 were used for varying lengths of time. They also used forty dogs, all of whom had been raised in the laboratory in their studies.

Their conclusions were that there was a certain definite individual tolerance to sulfite which varied in about a one to five ratio depending upon the individual, and that if the toxic dose was not exceeded, the ingestion of sulfur dioxide has no permanent deleterious effect upon the animal organism.

All sulfite, whether injected directly into the blood stream or given orally, is rapidly oxidized to sulfate and eliminated wholly via the kidneys. In general, they found that reasonable amounts of sulfur dioxide were not harmful and showed no lasting effect.

The American work, under the auspices of the Bureau of Chemistry, used fourteen human volunteers. These all received a uniform diet but were fed varying amounts of sodium sulfite, from 0.138 gram to 1.35 grams daily as SO_2, in the form of sodium bisulfite solution.

These workers found definite toxic symptoms, particularly apparent at the higher concentrations. These symptoms included nausea, headache, vomiting and diarrhoea. In

addition, they found a decrease in red cell and white cell counts of blood, a reduction in hemoglobin, a disturbance of phosphorous metabolism, and negative nitrogen balances extending after the termination of experimental feeding. There was also indication of loss of weight. They concluded that the use of sulfur dioxide for preservative purposes was not conducive to the best interests of the public health.

It may be pointed out here that the amounts of sulfur dioxide used in the diets of these experiments are all far in excess of any quantity that would normally be encountered. The accepted maximum quantity of sulfur dioxide in dried fruits is one-fifth of one per cent, corresponding to 2000 parts per million.

Inasmuch as 75% or more of this disappears or is oxidized during the process of preparation of the food for eating, it is readily apparent that the actual amount ingested is very small compared to the amount fed to the experimental groups above.

Further, the sulfur dioxide in dried fruits and vegetables exists in combination with various sugars, more or less firmly bound (3) and these compounds differ markedly from sodium bisulfite in their possible physiological effects.

Reasoning from the above discussion, it was decided for the purposes of the present study to use dehydrated apricots as the source of sulfur dioxide. In order that the quantity

necessary could be reduced to a reasonable amount, the sulfur dioxide content was raised to a high level.

METHODS AND PROCEDURES

Experimental Subjects

A total of sixteen dogs were used, both male and female. Ten of these were used for experimental purposes, the remaining six being used as controls. They were kept in individual cages. The amount of prepared dog food (Gaines Dog Meal) mixed with whole milk necessary to maintain the weight of each dog was determined before any experimental work was begun. They all were supplied with adequate water for their needs.

After normal values had been determined for all dogs, the experimental animals were fed the calculated quantity of sulfited apricots mixed with their food just prior to feeding. At intervals the following determinations were made on all dogs: red and white cell counts, differential counts, blood sugar, non-protein nitrogen of blood, plasma proteins, and calcium and phosphorous levels in blood.

In addition, ten volunteer male medical students received, during the experimental period, individually weighed portions of sulfited apricots, the amounts being calculated on the basis shown below under "experimental diet". Periodically, blood and urine samples were taken. Blood was examined for red and white cell counts, differential count, hemoglobin, glucose, non-protein nitrogen, plasma protein, serum calcium and serum

phosphorous. Determinations were made on urine of volume, reaction, reduction, albumin, calcium and phosphorus.

Experimental Diet

From diet lists (4) furnished by the United States Army, of their "B" ration, which is used under invasion conditions and contains the largest proportion of sulfur dioxide-treated foodstuffs of any of the various ration lists used, it was determined that the maximum amount of sulfur dioxide that any one man could receive per day was 0.6 milligram per kilogram of body weight. This is the sulfur dioxide content of the dehydrated fruit and vegetables before cooking. In the preparation process, these foodstuffs lose 75% of their sulfur dioxide content, either by oxidation or volatilization.

In some cases it is possible for a man under combat conditions to receive large amounts of sulfur dioxide through the medium of the fruit bars supplied as a part of the "C" and "K" rations. These are made of sulfited fruits, sugar, and added accessory food factors, and are not cooked before being consumed.

In view of the above considerations, it was decided that an amount of sulfur dioxide equivalent to three times the maximum amount available through a diet consisting exclusively of the "B" ration before preparation would be higher than it would be possible for any man to receive under actual field conditions.

The experimental diets were made up on the basis of daily intake of 1.8 milligrams SO_2 per kilogram per day. For a seventy-kilogram man, this amounts to 126.0 milligrams SO_2 per day. This is about the same amount as the minimum quantity used in the previous studies. (1), (2)

The dehydrated apricots used contained about 1500 parts per million of SO_2 when received at the laboratory. This would necessitate, if the apricots were used as received, the ingestion of about 85.0 grams of dried apricots by each man daily. In order to avoid the laxative and psychological effects of eating such a large quantity of apricots, it was decided to feed a smaller amount containing a higher concentration of sulfur dioxide.

After preliminary experiments, the following method was adopted for the preparation of the apricots used in the experimental diet:

One kilogram of the dried fruit, as received, was washed with three changes of hot water. Four liters of hot water and 100.0 grams of glucose were then added and the mixture allowed to stand over night. This was then homogenized in a Waring blender and the resulting thin paste placed in a large jar. Sulfur dioxide, from a cylinder of the liquified gas, was then run in through a glass tube reaching to the bottom of the jar, the current of gas being regulated so that practically all of it was absorbed. The mixture was agitated

at frequent intervals and the introduction of gas continued until it was judged that the material was saturated. The jar was then loosely covered and placed in the icebox over night. The following day the mixture was thoroughly stirred and the sulfur dioxide content determined by the method of Prater, Johnson and Pool. (3)

The procedure outlined gave a suspension of apricots containing from 12,000 to 15,000 parts per million of sulfur dioxide--equivalent to 45,000 to 60,000 p.p.m. on a dry basis. This suspension was unfit for consumption as such, having a vilely astringent taste and a suffocating odor.

In order to make the material sufficiently palatable, it was diluted with a paste made from apricots by the following method:

One kilogram of apricots were treated in the same manner as outlined above up to the point of being allowed to stand over night to soften. They were then cooked until tender. This usually took about one-half hour of gentle boiling. This fruit was then cooled and homogenized as before. The sulfur dioxide content was reduced by this procedure to a negligible value.

A mixture of the highly sulfited and cooked fruits was then made up to a uniform content of 4000 p.p.m. of SO_2.

This is the material used for the experimental feeding. The human volunteers received it as such in the manner outlined above, while the dogs received it mixed with their food.

Analytical Methods

Blood counts were made by standard clinical methods using hemocytometers.

Differential counts were made by thin smear. Wright's stain was used.

Hemoglobin was determined by use of a commercial Sahli-Haden apparatus, values being reported in grams per 100 milliliters. This was chosen because of the speed and simplicity of operation. It is recognized that this may not be the most accurate method of hemoglobin determination, but since in this study comparative values served the same purpose as absolute values, it was felt that the method was adequate for the purpose.

Blood sugar was determined on a zinc filtrate prepared according to the method of Somogyi. (6) The reducing value of the filtrate was then determined by the Shaffer, Hartmann-Somogyi (7) copper-iodimetric procedure.

Blood Non-protein Nitrogen was determined on a portion of the zinc filtrate by the method of Rinehart, Grondahl, and West. (8)

Plasma Protein was determined by the biuret method of Kingsley. (9)

Serum Calcium. The Clark and Collip (10) modification

of the Kramer and Tisdall method was used.

Serum Phosphorus was determined by a modification of the colorimetric procedure of Youngburg and Youngburg. (11) The only change made in this method was the substitution of Amidol for stannous chloride as the reducing agent.

Amidol reagent: 0.3 gram 2,4 diaminophenol-dihydrochloride (Eastman) and 3.0 grams sodium bisulfite A.R. Dissolve in 100 ml. distilled water.

Urine Reaction. Nitrazine paper was used.

Urine Albumin. Heller's nitric acid ring test was used.

Urine Sugar. Benedict's qualitative method was used.

Urine Calcium. The procedure used is as follows:

25.0 ml. of urine is placed in a 50 ml. centrifuge tube with a conical bottom. 2.0 ml. concentrated hydrochloric acid are added. Care is taken to see that all material is in solution, heat being used if necessary. Ammonium hydroxide is then added drop by drop until the mixture is just neutral to brom-cresol green (pH approximately 5.0), the mixture usually having a faint purplish color best observed against a white background. 2.0 ml. of 5% ammonium oxalate are then added, the solution is thoroughly stirred and allowed to stand for several hours. It is then centrifuged and the supernatant fluid poured off, being careful not to disturb the precipitate. The precipitate is washed twice with 25.0 ml. portions of 0.6% ammonia, stirring the precipitate with the wash liquid and centrifuging each time.

The precipitate is dissolved in 1.0 N sulfuric acid and heated in a water bath to about 90° C. The liberated oxalic acid is titrated with 0.05 N potassium permanganate.

1.0 ml. 0.05 N $KMnO_4$ = 1.0 mg. Ca

Urine Phosphorus. 5.0 ml. of urine are placed in a 25 x 200 mm. test tube, a glass bead and 2.0 ml. of concentrated nitric acid are added and the mixture heated over a micro-burner until pale amber color develops and all frothing has ceased. 2.0 ml. of concentrated sulfuric acid are then added by allowing the acid to flow down the side of the tube. Heat again until fumes of sulfur trioxide fill the tube and the liquid is almost colorless. Allow to cool and add three drops of 72% perchloric acid, and boil gently until colorless. When cold, the digest is transferred quantitatively to a 100 ml. volumetric flask and made up to volume with distilled water.

1.0 ml. of the diluted, oxidized urine is placed in a 100 ml. volumetric flask, and approximately 80 ml. of water are added. 5.0 ml. of a 2.5% solution of ammonium molybdate in 3.0 N sulfuric acid and 3.0 ml. of Amidol reducing agent are added, the mixture is shaken, made up to voluve with distilled water and allowed to stand twenty minutes. The color developed is read in a photo-electric colorimeter, and the phosphorous content is calculated from a comparison with a standard solution of potassium dihydrogen phosphate

containing 0.01 mg. of phosphorus per ml. The standard and unknown are prepared simultaneously, using the procedure above, except that the 2.5% ammonium molybdate solution is made up in 5 N sulfuric acid for the standard.

EXPERIMENTAL

Early in the work some of the men complained that the sulfited fruit was laxative in effect. For this reason it was decided that near the end of the experimental period some of them would be fed unsulfited apricots to determine whether this effect was due to the fruit or to sulfur dioxide. Notations have been made in the tables as to when this was done. Those men who had been fed sulfited fruit throughout were asked to give a blood sample about a month after the study terminated. The purpose was to determine the validity of some of the conclusions drawn from this study.

The researches of Williams and associates (12) on the structure of Vitamin B_1 and the role played by sulfite in the destruction of thiamine led to the belief that some of the effects attributed to sulfite ingestion might possibly be due to the in vivo destruction of thiamine with a consequent disturbance of the enzyme systems with which this vitamin is associated. It was originally intended that this be made a separate study, but the decision was made to make a few experiments to determine whether or not the addition of thiamine to the diet would correct any of the observed disturbances due to sulfur dioxide. These are indicated at the appropriate places in the tables.

On the following pages is shown the results of the experimental study.

TABLE I

Calculation of amount of SO_2 to be fed daily

Subject No.	Weight kg.	Amount SO_2 daily	Amount * apricots
Human			
#1H FVV	72.8 x 1.8 mg. =	132 mg. + 4 mg. =	33.0 G.
2H DP	72.7	131	32.8
4H GP	77.2	139	34.8
5H GH	72.7	131	32.8
6H TB	63.6	115	28.8
7H RR	70.5	127	31.8
8H JB	76.8	138	34.5
9H CHM	72.8	132	33.0
10H DD	81.8	147	36.8
Animal			
#2D f	18.2	33	8.3
3D m	10.2	18.5	4.7
4D m	13.9	25	6.3
5D m	7.7	14	3.5
6D m	24.6	43	10.8
7D f	17.3	31	7.8
8D m	12.3	22	5.5
9D f	22.7	41	10.3
11D f	19.5	32	8.0
15D m	13.0	24.5	6.2

* The apricots contained 4000 ppm. of SO_2. This is equivalent to 4.0 mg. per gram of fruit

KEY TO TABLES

Red Cell Counts	millions/cmm.	-	RBC
White Cell Counts		-	WBC
Hemoglobin	grams/100 ml.	-	Hhb
Non-protein nitrogen	mgs/100 ml.	-	NPN
Serum calcium	"	-	Ca
Serum phosphorus	"	-	P

TABLE II

Experimental determinations on blood of dogs.
Experimental group.

Date	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#2D								
Normal								
6-22	7.66	6750	15.8	63.2	31.9	5.3	12.2	3.3
7-10	7.62	11450	15.0	68.9	41.4	5.9	12.0	4.6
7-20	7.89	9850	16.2	81.6	36.1	6.0	10.6	3.3
Experimental								
10-16	5.75	8250	15.4	59.6	31.3	4.9	9.3	3.5
11-15	6.55	7600	17.5	59.2	33.8	5.8	11.7	6.0
12-19	6.79	8100	17.0	47.9	38.0	5.7	10.6	4.6
2-12*	6.39	12950	14.0	62.4	30.0	6.2	9.2	5.4
#3D								
Normal								
6-21	7.32	8100	16.5	82.4	49.4	6.2	17.1	5.0
7-12	6.23	8650	14.2	59.5	64.3	5.9	19.6	4.0
7-25	7.60	8750	15.0	64.8	48.2	5.5	10.1	5.1
Experimental								
10-17	6.82	8350	15.5	52.0	54.5	5.6	10.4	3.4
11-15	5.59	7650		56.5	58.1	5.4	15.1	4.3
12-19	4.82	8900	13.8	51.1	60.9	6.1	10.4	6.4
2-12*	5.75	15800	14.2	83.6	63.4	6.1	10.2	5.6
#4D								
Normal								
6-21	6.85	9300	15.3	85.6	37.4	6.2	12.1	5.1
7-10	7.29	9750	15.2	85.5	30.1	5.5	13.9	5.3
7-20	7.85	14550	15.2	89.0	43.4	5.7	9.8	4.4
Experimental								
10-18	5.58	9900	15.4	65.1	41.1	6.0	8.9	4.0
11-16	5.41	8750		60.6	35.2	6.1	10.7	5.3
12-22	6.03	12000	16.6	65.1	37.6	5.7	10.0	4.6
2-14	6.13	14500	14.7	65.5	36.1	6.3	9.2	4.9

*These dogs had been receiving yeast extract since 2-8.

TABLE III

Experimental determinations on blood of dogs.
Experimental group

Date	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#5D								
Normal								
6-23	6.78	17150	15.2	99.2	40.8	5.3	14.7	4.2
7-11	7.09	14450	14.6	86.0	50.8	5.5	11.9	4.2
7-24	7.03	14150	16.0	96.0	39.1	5.3	11.9	3.4
Experimental								
10-19	4.82	18850	14.2	63.3	36.2	5.6	9.6	4.6
11-16	5.70	16300	16.4	72.0	32.8	6.2	11.1	4.6
12-22	4.74	13600	13.4	74.6	34.7	5.8	10.1	5.2
2-14*	6.08	7350	15.1	79.6	32.9	5.5	10.7	3.9
#6D								
Normal								
6-28	7.75	15150	13.0	108.0	40.7	5.4	15.8	4.3
7-11	7.17	8300	13.4	90.4	52.6	5.8	14.1	4.7
7-24	6.83	14450	13.0	86.0	32.5	5.7	16.4	4.5
Experimental								
10-23	6.85	18200	15.2	45.7	37.8	6.7	9.1	4.1
11-20	6.18	12300	15.0	48.4	35.6	6.3	11.1	4.6
12-21	5.64	15050	16.4	57.5	53.5	5.5	12.1	5.4
2-15	6.23	12850	16.6	52.9	38.8	5.7	9.5	4.4
#7D								
Normal								
7-3	7.21	7850	15.8	76.0	36.6	5.3	11.1	4.4
7-17	8.60	7600	16.2	94.0	42.7	5.6	13.3	4.5
7-27	9.18	9900	17.2	74.2	43.6	5.3	12.3	4.9
Experimental								
10-24	6.31	9950	16.2	49.7	50.8	5.6	10.2	4.8
11-20	7.58	16400	17.8	42.1	41.8	6.2	10.6	5.0
1-6	6.19	10200	17.0	49.7	47.7	5.7	11.1	5.1
2-19	6.53	12450	17.0	47.0	31.8	5.9	12.2	6.5

* This dog had been receiving yeast extract since 2-8

TABLE IV

Experimental determinations on blood of dogs
Experimental group

Date	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#8D								
Normal								
6-27	6.50	15000	15.6	49.2	32.7	6.4	7.5	2.7
7-13	8.15	12700	16.5	86.8	34.8	5.7	12.0	3.8
7-26	8.59	10550	16.4	76.8	38.5	8.2	11.0	3.8
Experimental								
10-24	7.68	11400	17.3	54.2	57.1	6.3	17.4	4.3
11-21	6.78	8200	17.6	65.6	34.3	6.3	11.4	4.4
1-6	7.10	8200	17.2	50.6	34.5	6.0	15.9	5.6
2-19	7.78	13250	17.7	55.6	42.2	6.3	9.7	5.2
#9D								
Normal								
7-7	7.80	7600	17.7	68.4	42.6	5.9	12.6	4.9
7-19	8.42	6500	20.0	65.0	38.9	6.0	10.8	4.2
8-1	8.72	7350	18.4	75.6	32.1	5.6	9.7	4.2
Experimental								
10-25	6.61	7850	18.4	49.7	37.4	5.6	14.5	3.7
11-21	6.79	5100	18.8	45.2	36.2	5.3	10.2	4.8
1-8	6.65	8450	17.9	48.4	34.1	5.5	9.9	4.9
2-21	6.62	9350	16.9	49.3	36.1	5.4	8.7	5.5
#11D								
Normal								
6-30	8.33	9000	17.2	81.6	36.6	6.0	13.7	4.3
7-12	8.21	8550	17.6	81.2	42.8	6.3	12.2	4.1
7-25	9.12	8700	18.0	81.2	38.5	5.6	11.9	2.8
Experimental								
10-23	6.40	9050	16.6	51.8	39.3	6.2	12.3	4.6
11-27	6.92	8800	18.0	55.1	29.8	sample lost		
1-8	5.15	12000	17.2	55.1	37.9	6.3	12.4	4.9
2-21*	7.12	13700	17.0	61.9	36.9	6.1	13.3	4.6

* This dog had been receiving yeast extract since 2-8.

TABLE V

Experimental determinations on blood of dogs
Experimental group

Date	RBC	WBC	Hhb	Glucose	NPN	Plasma Prot.	Ca	P
			%	mg.%	mg.%	%	mg.%	mg.%
#13D Normal								
6-29	7.95	10800	15.8	75.2	38.6	6.3	12.2	4.2
7-13	7.45	10750	16.3	94.8	39.0	5.5	12.4	3.6
7-26	8.54	15500	17.2	85.2	38.5	6.5	10.6	4.3
Experimental								
9-28	5.19	18950	14.0		sample lost			
10-26	5.16	14650	14.0	56.9	37.4	5.0	11.5	4.5
11-27	5.43	10900	14.9	61.0	38.6	5.8	11.4	5.2

This dog had been suffering from an infected wound since early in September and was dropped from the work at this time.

TABLE VI

Experimental determinations on control dogs

Date	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#16D*								
8-19	6.56	19900	17.6	71.6	32.9	5.7	14.9	4.7
11-7	5.80	13600	16.0	51.0		5.8	11.3	4.0
12-12	5.29	11650	14.4	50.6	36.6	6.5	12.0	4.9
2-5	5.57	16750	15.3	52.4	37.6	5.8	11.6	5.7
#20D								
9-7	6.35	15200	16.6	54.7	46.7	5.3	12.9	4.1
11-7	7.20	7850	18.6	54.7	53.5	6.4	9.3	5.0
12-12	6.49	8100	17.5	68.2	38.4	5.9	10.3	4.8
2-13	6.95	8250	17.9	61.0	39.1	5.5	9.5	5.8
3-5	6.77	10200	17.0	53.8	36.2	5.6	12.6	5.4
#21D								
9-7	5.72	6850	16.1	56.8	52.6	6.2	10.0	4.2
11-8	6.99	8900	19.0	48.8	38.2	6.0	12.4	5.3
12-13	6.34	10850	17.2	58.8	44.8	6.1	11.7	4.7
2-7	6.75	11400	18.2	62.4	61.0	5.7	10.6	4.9
3-5	7.17	12700	18.2	60.6	36.8	6.1	11.8	6.1
#24D*								
10-10	6.02	15750	14.0	48.7	33.8	4.9	12.0	5.5
11-8	5.79	6550	14.0	55.6	34.6	5.9	12.9	6.6
12-14	5.92	8250	15.3	65.5	39.4	5.9	11.7	5.5
2-7	5.48	8450	13.4	71.4	39.8	5.9	11.9	6.4
#25D								
10-10	5.82	15100	14.8	59.2	43.4	5.2	11.7	4.3
11-9	5.82	16500	13.4	52.0	38.4	5.7	12.9	4.5
12-28	5.28	15300	14.0	54.2	39.2	5.3	13.6	5.3
2-8	5.28	17400	13.8	62.8	54.0	5.4	12.6	5.0
3-7	5.69	13450	16.5	62.4	38.4	6.2	12.5	5.5
#26D								
10-12	5.45	12300	13.8	60.6	41.2	5.0	10.0	3.6
11-9	6.00	10750	16.2	58.8	48.3	5.8	11.4	4.3
12-28	6.11	11450	16.4	51.5	48.0	5.8	11.6	4.3
2-8	6.27	10450	16.7	55.2	39.0	5.6	10.1	3.1
3-7	7.17	10750	16.4	55.1	38.9	5.9	11.6	4.1

*These dogs died from undetermined causes early in March

All of these dogs had yeast extract added to their food from 2-9 on.

TABLE VIA

Average of determinations on dog bloods

	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#2D								
Nor	7.72	9350	15.6	71.2	36.5	5.7	11.6	3.7
Exp	6.39	7475	15.9	57.3	33.3	5.6	10.2	4.9
#3D								
Nor	7.05	8500	15.2	68.9	54.0	5.8	15.3	4.0
Exp	5.74	10175	14.3	53.2	57.4	5.8	11.0	5.0
#4D								
Nor	7.33	11200	15.2	86.7	37.0	6.2	12.0	5.0
Exp	5.79	11290	15.6	64.0	37.5	6.0	13.0	4.7
#5D								
Nor	6.97	15250	15.5	93.4	45.6	5.3	12.8	3.9
Exp	5.83	14025	14.8	72.4	34.1	5.6	10.4	4.6
#6D								
Nor	7.25	12430	13.1	94.8	42.0	5.6	15.4	4.4
Exp	6.22	14600	15.8	51.1	41.4	6.0	10.4	4.6
#7D								
Nor	8.33	8440	16.5	81.4	41.0	5.3	12.2	4.6
Exp	6.65	12250	17.5	49.4	43.0	5.8	11.1	5.3
#8D								
Nor	7.74	12750	16.2	81.8	35.3	6.8	10.2	3.4
Exp	7.34	10260	17.5	56.5	42.0	6.2	13.8	4.9
#9D								
Nor	8.29	7150	18.7	69.7	38.4	5.8	11.0	4.4
Exp	6.67	7690	18.3	48.1	36.0	5.5	10.8	4.7
#11D								
Nor	8.55	8750	17.6	81.3	39.3	6.0	12.6	3.7
Exp	6.40	10890	17.2	56.0	38.0	6.2	12.7	4.7
#13D								
Nor	7.38	12910	15.8	84.7	38.7	6.7	11.6	4.0
Exp	5.26	14830	14.5	59.0	38.0	5.4	11.4	4.7
Controls								
#16D	5.80	15475	15.8	56.4	35.7	5.9	12.5	4.8
#20D	6.75	9725	17.5	58.5	38.8	5.7	10.9	5.0
#21D	6.59	10140	17.7	57.7	46.7	6.0	11.3	5.0
#24D	5.78	9750	14.2	60.3	36.9	5.7	12.1	6.0
#25D	5.58	15550	14.5	58.1	38.7	5.6	12.7	4.9
#26D	6.20	11140	16.1	56.2	43.1	5.6	10.9	3.9

Figure I

Average values of red cell counts on experimental dogs.

——— = normal

- - - - = on SO_2 diet

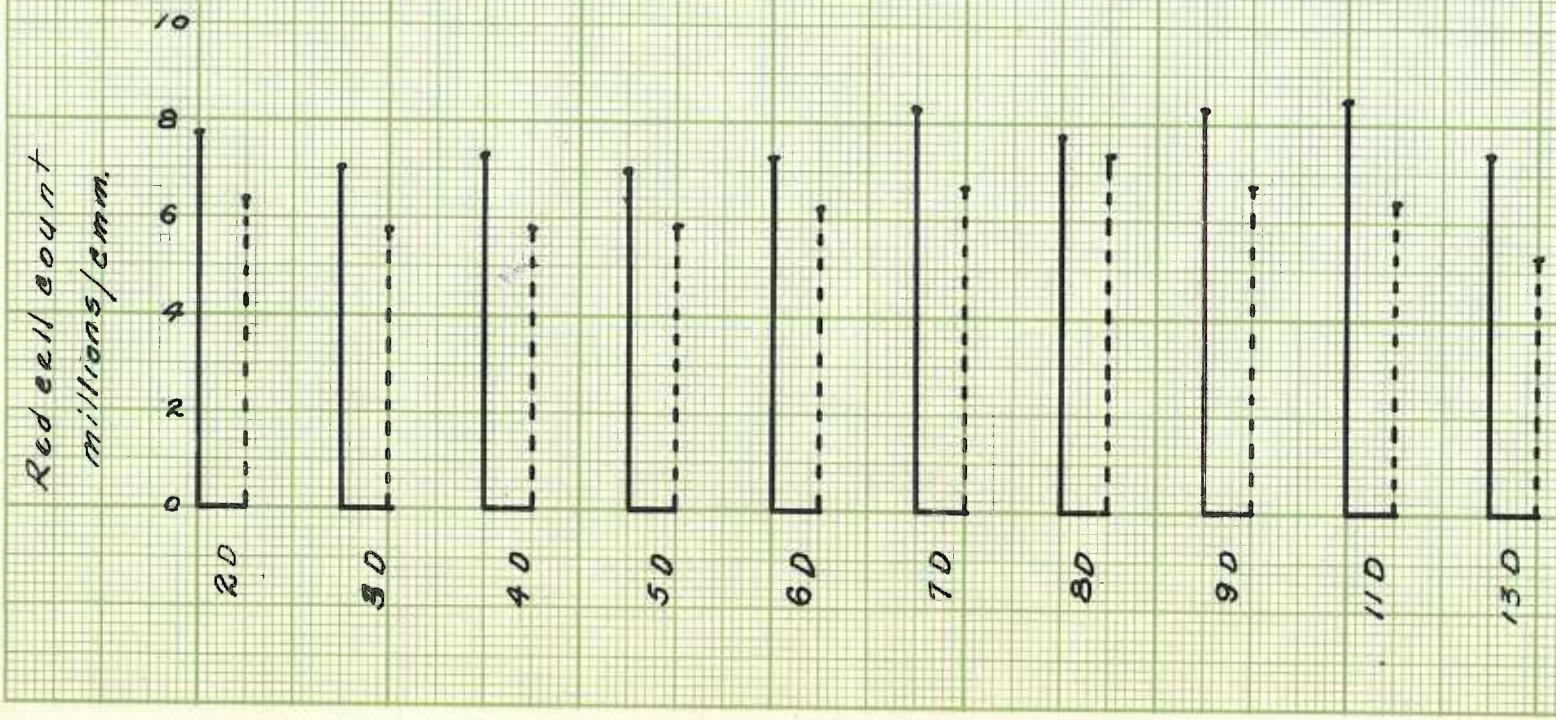

TABLE VII

Determinations on human bloods.

Date	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#1H Normal								
8-16	5.28	8450	16.4	90.8	36.1	6.3	18.4	5.0
9-11	4.55	6600	15.8	72.3	37.7	6.3	10.2	3.8
3-19[a]	5.52	6200	15.8	63.3	35.8	5.6	9.3	4.1
Experimental								
11-6	5.11		17.0	58.7	26.6	6.2	11.3	5.4
12-21	5.15	7550	16.4	66.0	38.4	5.8	9.8	4.6
1-24	5.22	7150	17.3	63.3	35.2	6.8	9.5	4.0
3-1	6.67	7050	15.2	73.7	36.5	6.0	9.7	5.6
#2H Normal								
8-16	5.59	7300	17.5	90.4	36.5	6.9	11.1	4.2
9-11	5.10	7100	16.2	79.6	38.5	6.3	9.8	3.9
1-3	4.75	6050	15.7	82.1	41.4	6.6	9.9	5.1
3-14[a]	5.32	6950	15.8	76.0	29.0	5.7	10.0	3.1
Experimental								
10-26	5.50	7000	18.0	70.5	31.1	6.0	9.8	3.6
12-5	5.41	8150	17.4	61.9	34.6	6.7	12.6	4.4
1-22	4.73	6050	15.8	68.9	35.4	6.1	10.8	3.6
2-28	6.02	5450	16.1	64.2	35.2	6.3	10.6	3.1
#4H Normal								
8-22	4.59	7850	15.8	71.4	34.5	5.9	11.6	3.1
9-20	5.35	9000	18.0		49.8	5.8	10.5	3.4
4-17				72.8				
Experimental								
10-25	5.09	7900	19.0	70.1	37.4	5.6	10.5	3.8
12-7	5.98	7650	17.3	73.7	38.2	6.0	11.2	4.2
1-4	5.25	11500	18.1	71.9	40.5	6.5	11.5	4.1
1-24	4.86	11150	17.2	64.6	32.8	6.6	12.4	4.1
2-28	5.62	7750	17.1	78.6	33.7	5.9	10.9	4.0
3-19	4.98	11200	16.8	66.0	26.7	6.0	10.1	3.8

a These men had not received SO_2 since 3-5

TABLE VIII

Determinations on human bloods.

Date	RBC	WBC	Hhb	Glucose	NPN	Plasma Prot.	Ca	P
			%	mg.%	mg.%	%	mg.%	mg.%
#5H Normal								
8-24	4.96	7100	15.6	80.6	40.9	5.6	11.0	4.7
10-11*	4.80	6850	14.0	62.8	41.9	6.0	11.2	3.8
4-18				89.1				
Experimental								
11-1	4.63	5300	15.0	72.3	42.3	5.7	11.7	4.0
11-30	4.97	5100	15.2	64.6	38.2	6.5	12.6	5.6
1-31	5.14	4800	15.3	72.0	40.4	6.0	10.9	4.1
2-22	4.81	5400	14.9	70.1	34.8	5.8	9.6	4.9
3-14	4.97	5500	15.2	71.9	36.3	5.9	10.5	3.6
#6H Normal								
8-24	4.85	6600	15.2	78.8	35.6	6.4	10.8	4.0
10-11	5.10	6800	15.8	61.9	39.5	6.2	9.6	3.8
3-15[a]	5.15	5650	15.6	79.1	33.2	6.5	10.3	3.3
Experimental								
11-1	5.20	7350	16.4	65.5	38.5	5.7	10.1	4.0
11-30	4.50	7050	16.2	69.2	36.2	5.9	11.7	4.0
1-31	5.21	7100	16.2	65.1	34.0	6.3	9.9	4.0
2-22	4.75	8250	15.2	66.0	35.6	5.9	10.5	4.0
#7H Normal								
8-29	4.83	8300	15.6	71.0	38.7	6.2	12.0	3.0
9-19	5.07	7850	17.1		41.1	6.5	12.9	3.8
Experimental								
10-31	4.83	7100	17.0	57.8	34.8	6.2	12.9	3.5
11-28	4.80	8900	15.4	75.5	37.4	6.3	10.5	4.2
1-28	5.33	10900	16.2	79.1	36.7	6.7	10.8	4.2
2-26	5.05	7550	16.5	73.7	32.0	6.0	11.1	4.2
3-12	4.92	8050	15.2	74.6	35.9	6.2	10.2	3.8

* This man had given a blood transfusion two days before the sample was taken

a These men had not been receiving SO_2 since 3-5

TABLE IX

Determinations on human bloods.

Date	RBC	WBC	Hhb	Glucose	NPN	Plasma Prot.	Ca	P
			%	mg.%	mg.%	%	mg.%	mg.%
#8H Normal								
8-29	5.57	7350	17.0	73.2	36.1	7.2	24.6	3.8
9-12	5.24	6800	17.3		32.7	6.9	10.2	3.4
3-12[a]	5.61	9250	16.0	81.4	30.4	6.7	9.3	4.1
Experimental								
11-28	5.65	7850	18.5	62.0	37.0	7.3	11.9	4.4
1-28	5.81	6400	16.5	75.0*	35.5	7.3	11.5	5.1
2-26	5.38	7350	15.1	57.8	30.0	6.5	11.0	5.1
#9H Normal								
8-30	4.77	7700	15.2	67.2	44.6	6.0	11.3	4.8
9-13	5.19	8500	17.0		33.7		11.5	4.6
3-21[b]	4.88	7100	15.7	70.7	34.4	5.9	9.5	4.1
Experimental								
11-2	4.98	8850	16.6	61.0	37.4	5.6	13.2	4.6
12-6	4.84	6600	16.2	56.1	38.3	6.0	9.8	5.2
1-25	4.66	7000	16.3	60.6	33.7	6.3	11.5	5.5
#10H Normal								
8-30	5.40	9200	17.5	76.8	40.2	6.3	12.7	4.3
9-13	5.44	7200	17.6		41.1		11.1	3.7
1-4	4.92	8250	17.8	89.4	34.2	6.8	11.6	4.9
4-19				84.1				
Experimental								
11-2	5.54	9900	17.6	71.6	38.5	6.3	8.5	4.4
12-6	5.84	10500	17.6	61.9	37.4	6.9	10.2	4.9
2-1	5.82	8900	17.4	73.7	25.1	6.5	11.3	5.4
2-26	5.11	7800	16.5	52.4	47.2	6.1	17.3	5.1
3-15	5.41	7850	16.7	66.0	23.5	6.4	10.3	3.9

* This man had not had SO_2 for two days.

a This man had not been receiving SO_2 since 3-5

b This man was ill with scarlet fever from 2-23 to3-10

TABLE IXA

Average of determinations on human bloods

	RBC	WBC	Hhb %	Glucose mg.%	NPN mg.%	Plasma Prot. %	Ca mg.%	P mg.%
#1H								
Nor	5.12	7080	16.0	72.1	36.5	6.1	12.6	4.3
Exp	5.04	7270	16.6	65.2	34.2	6.2	10.1	4.9
#2H								
Nor	5.19	6850	16.3	82.0	36.4	6.4	10.2	4.1
Exp	5.16	6660	16.8	66.4	34.1	6.3	10.9	3.7
#4H								
Nor	4.97	8425	16.9	72.1	42.1	5.9	11.1	3.3
Exp	5.36	9525	17.6	70.8	34.9	6.1	11.3	4.0
#5H								
Nor	4.88	6975	14.8	77.5	41.4	5.8	11.1	4.3
Exp	4.90	5220	15.1	70.2	38.4	6.0	11.1	4.4
#6H								
Nor	5.03	6350	15.5	76.6	36.1	6.4	10.3	3.7
Exp	4.91	7440	16.0	66.6	36.1	5.8	10.6	4.0
#7H								
Nor	4.95	8075	16.4	71.0	39.9	6.4	12.4	3.4
Exp	4.99	8500	16.1	71.9	35.4	6.3	11.1	4.0
#8H								
Nor	5.47	7800	16.8	77.8	33.1	6.8	14.7	3.8
Exp	5.61	7200	16.6	64.9	34.2	7.0	11.5	4.9
#9H								
Nor	4.95	8435	16.0	68.8	37.6	5.9	10.8	4.5
Exp	4.83	7485	16.4	59.2	36.4	6.0	11.5	5.1
#10H								
Nor	5.24	8220	17.6	83.4	38.4	6.5	11.8	4.3
Exp	5.54	8990	17.2	65.1	33.9	6.4	11.5	4.7

Figure III

Average of blood glucose levels in human subjects

N = normal values

E = experimental values on SO_2 diet

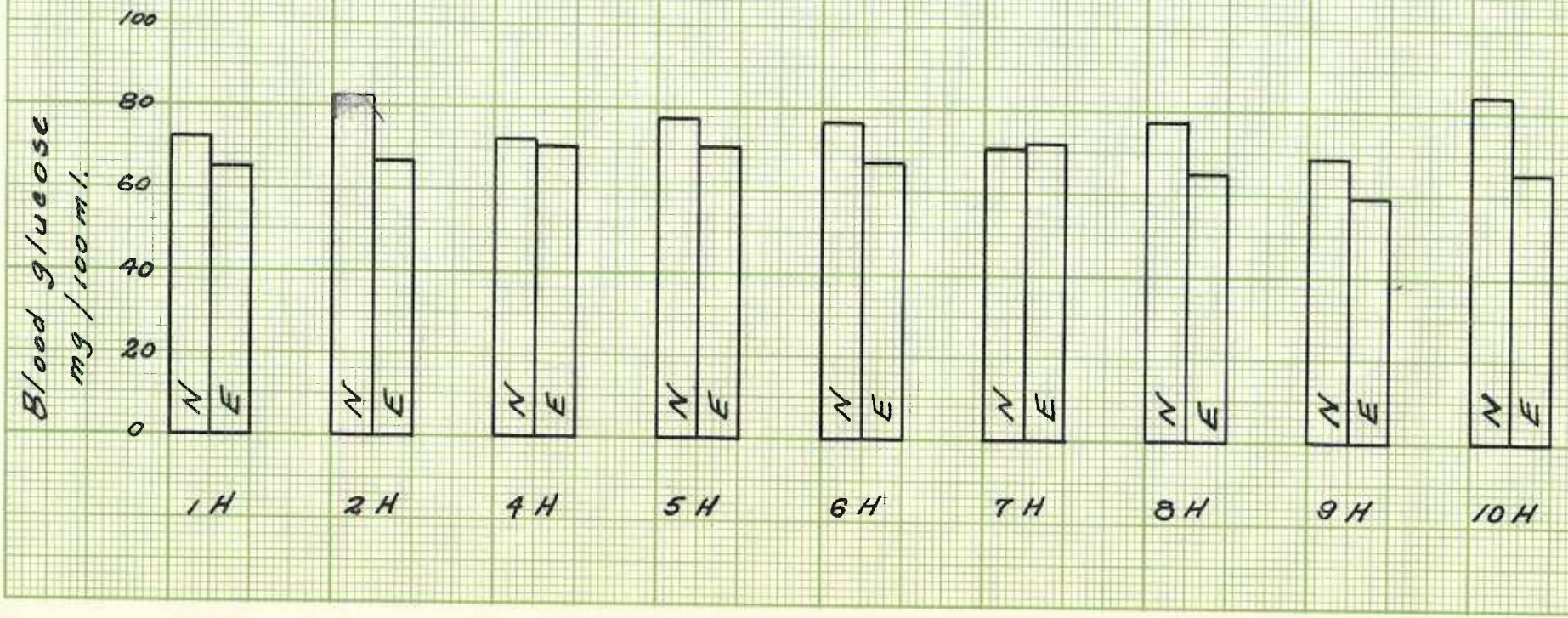

TABLE X

Determinations on human urine.

Date	Volume ml.	Reaction	Calcium mg./100 ml.	Phosphorous mg./100 ml.
#1H Normal				
8-31	2000	Acid	1.44	71.52
10-6	2130	Acid	4.59	33.3
1-7	2175	Acid	8.58	30.4
1-8	1615	Acid	6.82	35.9
Experimental				
10-27	2500	Acid	3.84	37.9
12-23	2450	Acid	5.52	35.8
1-27	1690	Acid	7.53	34.1
3-2	2600	Acid	4.82	29.9
3-12	2290	Acid	5.81	24.7
#2H Normal				
8-30	1300	Acid	15.80	70.6
1-4	1260	Acid	6.38	37.2
1-5	960	Acid	8.28	36.8
1-6	1315	Acid	12.36	38.4
Experimental				
10-27	1200	Acid	14.96	46.8
12-8	1075	Acid	15.60	48.6
1-26	1550	Acid	2.42	37.0
2-26	1580	Acid	10.96	33.1
3-16	1025	Acid	11.71	33.2
#4H Normal				
9-11	800	Alk	3.24	73.4
Experimental				
10-25	1375	Alk	6.12	47.2
12-6	1130	Alk	8.96	48.6
1-2	715	Alk	26.18	43.7
1-24	1150	Alk	14.39	41.2
3-7	1050	Alk	18.56	37.6
3-12	870	Alk	28.82	42.4

TABLE XI

Determinations on human urine.

Date	Volume ml.	Reaction	Calcium mg./100 ml.	Phosphorous mg./100 ml.
#5H Normal				
8-24	1800	Acid	18.52	46.2
1-4	1500	Acid	20.36	41.7
1-5	1735	Acid	5.32	35.8
1-6	1775	Acid	2.11	35.7
Experimental				
10-31	1550	Acid	16.82	44.2
12-7	1045	Acid	15.64	46.3
2-1	1345	Acid	15.20	38.7
2-6	1340	Acid	12.48	32.5
3-15	1085	Acid	21.42	31.6
#6H Normal				
8-28	950	Alk	21.72	53.2
1-5	965	Alk	14.64	38.4
1-6	1315	Alk	6.95	38.7
Experimental				
10-10	1200	Alk	22.76	47.4
10-27	950	Alk	20.84	48.4
11-28	1480	Alk	12.76	42.6
1-31	1130	Alk	23.61	39.7
2-24	1370	Alk	8.02	36.7
3-16	1500	Alk	13.25	36.2
#7H Normal				
9-19	1150	Neut	29.52	47.7
1-4	1950	Acid	24.51	37.2
1-5	1750	Acid	14.70	34.2
1-6	1000	Acid	2.86	39.9
Experimental				
10-31	1600	Acid	26.56	47.5
12-23	1490	Acid	20.72	50.5
2-7	1490	Acid	18.74	38.9
3-14	995	Acid	20.04	39.1

TABLE XII

Determinations on human urine.

Date	Volume ml.	Reaction	Calcium mg./100 ml.	Phosphorous mg./100 ml.
#8H Normal				
8-30	1600	Acid	1.76	74.8
1-5	775	Acid	28.42	43.8
1-6	840	Acid	39.30	40.0
1-7	640	Acid	15.79	40.0
Experimental				
9-14	1050	Acid	1.97	46.2
12-5	1200	Neut	2.16	45.9
2-23	955	Acid	6.53	39.9
3-12	715	Acid	34.72	40.3
#9H Normal				
8-29	1350	Neut	22.76	70.1
1-5	1770	Acid	13.86	38.4
1-6	860	Neut	14.56	36.8
1-7	1450	Alk	4.80	34.9
Experimental				
10-29	1150	Alk	18.38	52.6
12-12	1330	Alk	12.88	50.9
2-5	1305	Neut	12.20	40.2
#10H Normal				
8-29	1725	Neut	19.72	62.6
1-4	1840	Neut	10.24	38.1
1-5	1510	Alk	4.14	38.8
1-6	2100	Neut	9.86	35.9
Experimental				
10-28	1600	Neut	20.53	49.3
12-12	1640	Alk	21.84	47.4

None of the urines examined showed more than slight traces of albumin or sugar.

DISCUSSION

From the foregoing tabulations of the experimental results, it is possible to determine the following:

Red cell counts are uniformly lowered in the experimental dogs, while the control dogs do not show a similar lowering. The human subjects do not have any uniform variation in erythrocyte counts. This difference can possibly be explained on the basis that there was not the uniformity of control over the diet etc. of the human subjects that was obtained in the case of the experimental animals.

There is not an apparent regularity in the variations of leucocyte counts, hemoglobin, non-protein nitrogen, plasma proteins, serum calcium or serum phosphorous in either animals or men.

Blood sugar is lowered to a significant extent in the experimental animals and some of the human volunteers. The control animals show no tendency of this kind. It may be mentioned that those men who habitually drink milk do not show this lowering of blood sugar levels. The reason for this is not known.

Those men who were removed from the sulfite diet before the termination of the experimental period show a marked increase in blood sugar on the non-sulfite diet. Those men whose blood sugar levels were determined approximately four

weeks after the termination of the experimental feeding likewise show increased values for blood glucose. It is felt that this phenomenon of lowered levels of blood sugar is directly related to the sulfite intake. This effect disappears within one or two days at most after removal of sulfite from the diet.

Four of the experimental dogs were fed yeast extract during the latter part of the experimental period. The quantity fed was calculated on the basis of the thiamine content of the extract, each dog to receive 2.0 milligrams of thiamine daily. All of these animals showed marked increases in blood sugar values, and three of them showed increased red cell counts. Apparently, the addition of a moderate amount of thiamine to the diet will in a large measure counteract the effects of sulfur dioxide-treated foodstuffs.

The analyses of human urines show that there is an apparent disturbance of calcium and phosphorous metabolism. The variations in calcium are particularly marked. The exact nature of this effect is not known, but the probable explanation is that the partition of calcium between feces and urine is subject to wide variation. The tendency of urine phosphorous to decrease on sulfite diets probably has a similar explanation, although the work of Wiley (2) cited previously tended to show that this effect is of a more profound nature.

SUMMARY

The experimental results confirmed previous work in the following:

1. There is a reduction in red cell counts of the experimental subjects.

2. There is a disturbance of phosphorus metabolism.

Contrary to the findings of previous investigations, this study indicated that:

1. Albuminuria was not present.

2. White cell counts did not change significantly.

3. No toxic symptoms developed in the human subjects.

4. No evidence of impaired nitrogen metabolism was found.

The following had not previously been reported:

1. There is a disturbance of calcium metabolism.

2. Blood sugar levels are lowered to a significant degree under the conditions of this study.

3. The addition of thiamine to the experimental diet counteracts the observed effects to a great degree.

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