THE ACTION OF CERTAIN SULPHUR COMPOUNDS ON METABOLISM AND EXCRETION

BY CHARLES O. JONES, M.D., CH.B., M.R.C.S., L.R.C.P.

From the Laboratory of Bio-Chemistry, University of Liverpool

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The Sulphates. There has recently been much dispute as to whether the soluble sulphates exhibit their well-known purgative effects when administered otherwise than by the alimentary tract. In the present experiments it has been shown that no purgative action follows administration of sodium sulphate hypodermically; but there is, however, a slight increase in the quantity of the faeces, this increase not commencing until twenty-four hours after administration and terminating about twelve hours later. These faeces contain sulphates in much larger amounts than at other times. Several noteworthy metabolic changes were also noticed as the result of hypodermic injection of sulphates.

The animals used in all experiments were rabbits. The food was carefully weighed daily. The urine, and in a few instances the faeces also, were collected and subjected to examination and estimation of the various constituents. Sulphates were estimated by Folin's method. Five c.c. of the urine was used in each instance, and after acidulation with dilute hydrochloric acid, barium chloride solution was added drop by drop. After standing all night the precipitate was filtered off, ignited and weighed.

Five c.c. of the urine was heated with two per cent. hydrochloric acid for an hour, and to it barium chloride solution was added drop by drop; the precipitate after standing two hours was filtered, ignited and weighed. The difference between these two determinations gave the amount of sulphates combined with aromatic substances. Urea was estimated by Little's nitrometer; nitrogen by Kjeldahl's method; the other constituents in the urine were estimated by the usual methods.

It is interesting to note that the hypodermic injection of sodium sulphate does not affect the sum total of metabolic range to any great extent. Following small doses (up to one gram) the animal continued to gain weight, very much as before, even after somewhat prolonged administration. Large doses seemed to lessen the gain, but even these animals continued to gain weight slowly.

Very small doses seemed to cause a slight increase of the moisture, and to a less extent of the quantity of the faeces, but as the dose increased the faeces became less in quantity and contained less moisture.

A small hypodermic injection (0.5 gram) was followed by a very marked increase in the volume of urine excreted; this amounted to about three times the normal volume. Each constituent of the urine was markedly increased to about three times the normal amount, and in the case of the inorganic sulphates there was usually a still greater increase.

The following day the volume of urine and amount of each of its constituents is slightly diminished, and in from five to six days the urine has returned to its normal condition.

It is remarkable that the weight of sulphates excreted during these six days is much greater than the sum of the amount contained in the food and the amount given in the hypodermic injection.

Urinary volume in c.c.	Urea	Inorganic sulphates	Aromatic sulphates	Chlorides	Phos- phates	Ammonia	Uric acid	Nitrogen in other forms
150	0.486	0.506	0.045	0.0942	0.0410	10.0597	0.0782	0.0161
154	1.709	2.156	0.154	0.3719	—	0.2042	0.2232	0.170
138	1.366	1.476	—	0.4540	0.0927	0.0844	0.0579	—
125	0.825	0.925	0.1625	0.2131	—	0.0642	—	—
50	0.351	0.780	0.0225	0.1616	0.0432	0.0399	—	—

As the amount of sulphate is increased, the marked diuresis disappears; the amount of sulphate necessary is about two grams, but varies considerably. The most noteworthy feature of the urine during the twenty-four hours following a large injection is the increased excretion of inorganic sulphate accompanied by a diminished excretion of aromatic sulphate; this becomes more marked as the amount of sulphate is increased. The volume of urine and weight of its other constituents are not altered during this first twenty-four hours. The ratio of aromatic sulphate to inorganic diminishes from one to ten till it becomes one to sixty-two, the aromatic sulphates being one-twentieth their normal quantity. The amount of sulphate excreted in the faeces during the same twenty-four hours was about normal, being one-fifth that excreted in the urine.

The following day the diuretic action took place; the volume of urine was increased over three times the normal amount. Large quantities of inorganic sulphates were got rid of, and the aromatic sulphates were greatly increased in amount to about eight times the normal amount. The ratio of organic to inorganic sulphates was now

one to four. Urea was not as large as it should be in proportion to the amount of the other constituents, but these were increased in proportion to the increase in urinary volume.

Irritation of the renal cells was evidently present, there being casts in the urine, both epithelial, and more especially granular. These casts were first noted after one gram of sulphate had been given, so that it probably has a very irritating action on the renal cells, and probably on other living cells. Insoluble phosphates of calcium and magnesium were invariably present about forty-eight hours after an injection of sulphates; with larger doses calcium carbonate and calcium oxalate were also found.

Urinary volume in c.c.	Urea	Inorganic sulphates	Organic sulphates	Chlorides	Phosphates	Ammonia	Uric Acid
50	0.486	0.506	0.045	0.0942	0.0410	0.0597	0.0782
63	0.605	1.121	0.0063	0.1541	0.0759	0.0525	0.0822
162	0.907	1.247	0.3402	—	—	0.1157	0.0656
130	1.728	1.092	0.2080	—	—	0.0322	0.0692

During the first twenty-four hours the excretion of sulphates in the faeces was about normal; during the next twenty-four hours corresponding to the diuretic action shown in the above table there was a sudden increase in the amount of sulphate excreted in the faeces. The amount excreted in this way was three times the normal amount, and corresponded roughly to the increase in the urine. The amount normally excreted in the faeces is about one-fifth of the amount given out in the urine. After the hypodermic injection the faecal excretion of sulphate became seven-ninths of the urinary excretion. The following day the excretion of sulphates fell again to normal, and continued so.

Larger doses hypodermically caused exaggeration of these symptoms; two or more days now supervened before the diuretic effects were seen. The aromatic sulphate excretion became exceedingly small, being one two-hundredth the amount of the inorganic. A certain quantity of sulphate seemed to be required to be excreted before the rise in metabolism occurred, and the greater the quantity injected the longer time interval was required before the concentration of sulphates in the blood had been brought down to such a point that a metabolic rise occurred and the animal commenced to return to its normal condition.

With the large doses, casts almost entirely granular were always found in the urine in large quantities; such a marked irritation of the renal cells would point to a very irritating action by the sulphates.

Phosphates of calcium and magnesium were present after each injection. With small doses of sulphates, carbonate and oxalate of calcium were present, but these two salts increased very greatly in amount as the dose of sulphate was increased.

Experiments in Autolysis. An attempt was made by the aid of autolysis to find out if sodium sulphate had any effect on the metabolic changes in living cells. For this purpose 10 grams of finely-minced fresh liver were used in each experiment, using every aseptic precaution; to this minced liver was added fifty cubic centimetres of sterile distilled water, two cubic centimetres of toluol, and various quantities of sodium sulphate. A control without any sulphate was also made. All the preparations were placed in an incubator at thirty-eight degrees Centigrade for three days. Each was then boiled, filtered, and the filtrate and solid portion were then estimated separately for total nitrogen by Kjeldahl's method.

It was found that there was slightly more autolysis in the control, but not more than was found to be present when any other salt was used instead of the sulphate.

From these results it would seem that sodium sulphate in small amounts has a stimulating effect on the body cells, especially those of the renal and, to a less extent, of the intestinal epithelium. There is, however, a point above which this stimulant action becomes less marked, this being probably due to the concentration of sulphates in the blood being too high. This high concentration limits to a more or less extent, according to the amount of sulphate present, the normal interchange of products between the blood stream and the individual body cells. This is shown by, firstly, the greatly diminished excretion of aromatic sulphates from the cells, and the absence of the strong metabolic stimulus shown by the marked diuresis which follows small doses.

During this first twenty-four hours after a large dose, sulphate, almost entirely as inorganic sulphate, is being excreted, and when the blood concentration of sulphate is reduced to a certain point there is an immediate metabolic stimulus and the marked diuretic effect is seen, and at the same time a greatly increased amount of aromatic sulphate is thrown out, showing definitely that the sulphate has not had a poisonous effect on the cells, but rather has produced a barrier between the blood and the living tissue cells. The action of sodium sulphate hypodermically on the intestine is very similar but much less marked, for when the sulphate is in high concentration in the blood there seems to be little

or no power to get rid of it through the intestinal epithelium; but when the kidney has reduced the concentration to the necessary point, there is then a large increase in the sulphate excreted through the intestinal tract. It must, however, be pointed out that the weight of sulphate excreted by the intestinal tract amounts only to about 0·8 gram, and as the kidney is excreting more than three times its normal volume of water during this day, it is hardly strange that no marked purgative action follows *hypodermic* injection of sodium sulphate, and, as mentioned above, more sulphate injected into the blood represents more work for the renal epithelium to do before the point is reached when the marked diuresis occurs. An increased amount of sulphate is simultaneously given out in the faeces, and this increased intestinal excretion only lasts for a few hours, the excretion next day by that route being normal.

The marked irritation of the renal epithelium as shown by the presence of casts, both epithelial and granular, in the urine after the hypodermic injection of sodium sulphate, is important. This was first noticed when less than one gram had been injected. As the dose was increased, casts became more and more abundant. As malted drinks contain large quantities of sulphates, and as those addicted to such drinks seem especially prone to such diseases as chronic renal disease and cirrhosis of the liver, both diseases probably due to some toxic irritation, it seems possible that the sulphates present may be at least one cause of such conditions, and not the alcohol alone, which is not present in very large quantities. Lastly, the presence of calcium phosphate, carbonate and oxalate found in the urine after injection of sulphates, and probably due to some interference with metabolism, is curious in that these salts are very often present in the urine of drinkers of malted liquor.

The Sulphites. The metabolic action of sodium sulphite administered hypodermically in moderate doses on animals is as yet unknown. The effect of a poisonous dose is to kill by depressing the heart and circulation generally, and also the respiration. The same methods were adopted as in the case of the sulphates, the solution of the drug, freshly prepared, being injected hypodermically in all cases.

Hyposulphite of sodium was first used. It was found that after hypodermic injection there followed a marked diuresis, the urinary volume being about three times the normal amount and all the urinary constituents being increased. Casts, both epithelial and granular, were present, and more marked than after similar doses of sulphate, and there

followed even larger quantities of phosphates, carbonate and oxalate of calcium, than after sulphate injection.

It will be seen, therefore, that sodium hyposulphite acts very similarly to sodium sulphate, and is so rapidly oxidised in contact with the body cells that the effects may be considered as due to sulphates.

Sulphite of sodium was next investigated. The following day, after the hypodermic injection of 0·1 gram, there was an increase in the urinary volume, and also in the urinary constituents, an effect resembling that due to sulphates, and probably due to a portion of the sulphite being oxidised to sulphate. With this action there was found a large increase in the normal colouring matter in the urine, the presence of granular casts, and also of insoluble calcium salts. These effects are, however, not found after a dose of sulphate corresponding to that which the whole of the injected sulphite would yield on oxidation, so they must, in part at least, be accredited to unoxidised sulphite action in the blood. The following day the urine was diminished; there was still excess of colouring matter, and insoluble calcium salts, mostly phosphatic.

On the third day the volume of urine was greatly increased, a large amount of sulphates were thrown out, together with a great excess of calcium and magnesium salts—chiefly carbonate with some phosphates and oxalates—and a large number of granular casts were present.

Amount	Urea	Inorganic sulphate	Organic sulphate	Chlorides	Phosphate	Ammonia	Uric acid
45	0·398	0·386	0·048	0·1246	0·0211	0·0407	0·0510
90	1·368	0·426	0·054	0·1820	0·0810	0·0474	0·0623
24	0·392	0·3321	0·031	0·1210	0·0341	0·0418	0·1011
140	1·706	1·218	0·162	0·2600	0·0711	0·879	0·0631

As the dose of sodium sulphite is increased the stimulant action of the sulphate produced from it by oxidation is more and more obscured, and the depressent action of the unaltered sulphite becomes more marked. Thus, after injection of half a gram of the sulphite hypodermically, during the following three days only thirty cubic centimetres of urine were passed. This amount is much less than is normally passed in one day. There was, moreover, no increase in the amount of sulphates thrown out, showing that the sulphite had an inhibiting action on the oxidation processes which would change it into sulphate. At the same time, a great excess of the ordinary urinary pigment was present, together with a very large quantity of insoluble salts, so that the urine was milky in

colour and consistency. This salt was found to be almost entirely calcium carbonate, with a smaller quantity of calcium phosphate and oxalate, accompanied by many granular casts. On the fourth day sulphates commenced to be excreted above the normal amount, the top level of sulphate excretion with this amount of dose being attained on the sixth day after injection.

A still larger hypodermic injection was then given, amounting to one gram of the sulphite. No urine at all was passed during the following two days. The following day a quantity just above normal, of a thick, cloudy, dark orange-coloured urine, was passed. This orange colour was found to be due to the normal urinary pigment in excess. All the constituents of the urine, including sulphates, were about normal. Casts were present in large quantities. The thickness and cloudiness of the urine was due to carbonate of calcium, with some phosphate and oxalate, and also traces of magnesium salts.

The following day the animal cells had begun to overcome the depressing action of the sulphite, for although the urine was not increased in amount the urea began to rise in amount, as also the sulphates. The following day there was no urine again, and this was next followed by a rapid rise in the amount of urea formed and the amount of sulphate excreted, showing that the sulphite had been oxidised and excreted.

Amount	Urea	Inorganic sulphate	Organic sulphate	Ammonia	Uric acid
Nil	—	—	—	—	—
Nil	—	—	—	—	—
60	0.408	0.305	0.012	0.0163	0.0288
63	0.931	0.882	0.246	0.0083	—
Nil	—	—	—	—	—
87	0.731	1.566	0.139	—	—
160	1.088	1.104	0.112	0.0487	0.0127

In these hypodermic injections of sodium sulphite the enormous excretion of calcium carbonate, and to a less extent calcium phosphate and oxalate, was a most noticeable feature; this deposit, after settling, was about half the bulk of the whole urinary volume. The quantitative estimation of these salts will be found in the section on sodium sulphide, which was also found to produce them in large amount.

It was found that different animals differed very much in their reaction to sodium sulphite; in other words, they differed in their oxidising powers. The previous rabbit had reacted strongly to one gram

of sodium sulphite. The same quantity hypodermically injected into a fresh rabbit produced death in half an hour, with the usual well-known symptoms, viz., depression of the circulation and respiration; while a third rabbit oxidised half a gram of sulphite so quickly that the symptoms were similar to sulphate injection. Even one gram in this animal produced only similar symptoms to that shown by the first rabbit after one-tenth of a gram.

Experiments in Autolysis. The effects of hyposulphites and sulphites of sodium were then tried on autolysis. Ten grams of finely minced liver were added to fifty cubic centimetres of sterile distilled water, using every aseptic precaution. Two cubic centimetres of toluol were added. This served as a control. To similar preparations various amounts of hyposulphite and sulphite were added, and the whole placed in an incubator at thirty-eight degrees Centigrade for seventy-two hours. Each was then boiled, filtered, and total nitrogen in the liquid and solid portions each separately estimated by Kjeldahl's method.

It was found that hyposulphite had no appreciable effect. Sulphite, however, was found to be different; up to a certain amount it had no effect on the rate of autolysis, but when that point was passed there was an almost immediate stoppage of all autolysis.

	Nitrogen in soluble part	Nitrogen in insoluble part
Normal liver, boiled before autolysis commenced	0.0381	0.2836
Normal liver autolysis	0.1671	0.1532
Liver with sulphite	0.0428	0.2762

It would therefore appear that sulphite has an inhibiting effect on autolysis, due probably to combination with the enzymes of the cell. But as the effect of sulphite on an animal is very variable, depending on the oxidation powers of that animal, so that the effect of sulphite on autolysis was found to be very different in different tissues. In some liver tissue, ten grams always being used, as small a quantity as two decigrams was sufficient to arrest all autolysis, while in another liver five decigrams were necessary, and in some cases very much larger quantities were required; but in all cases there is a fixed point which may be called the oxidation limit, any sulphite above which being added will fix the ferments and cause the stoppage of all metabolic changes.

From these experiments it appears that when the sulphite is too great for the oxidation powers of the cells, it immediately combines with the ferments and in this way stops all metabolic tissue changes, and

death results. But this result does not show us whether the sulphite becomes attached to the cell and is there attacked, or whether the oxidising ferments attach themselves to the sulphite, so that it is only when the sulphite is in excess that it becomes attached to the cell.

A rat was given a large dose of sulphite of sodium hypodermically, and a few minutes afterwards it was killed, as well as another untreated rat to serve as a control. On submitting the livers to autolysis for twenty-four hours no difference was found in the rate of autolysis; the sulphite liver, in fact, had progressed further than the control, which points to the fact that the sulphite becomes anchored in the body to oxidising ferments.

As a further test, a large dose of sulphite (one gram) was given to a rat, and an hour and a half afterwards the rat was killed, its liver, spleen and kidneys removed, finely minced, and placed in a small flask connected with glass tubing to another small flask containing a weak solution of barium chloride. Two per cent. hydrochloric acid was added to the tissues, which were then heated. Not the slightest trace of barium sulphite was produced, nor in substituting a weak solution of copper sulphate was any insoluble copper sulphite produced, which tends to show that the sulphite does not long exist as such in the living cells.

From these results it appears that sodium sulphite exerts two distinct actions in the animal body. One action is due to sodium sulphate into which part of it is almost immediately oxidised, and the other action is due to excess of free sulphite which the body is unable to cope with. The proportion of these two depend in the first place on the dosage; a small dose acting almost entirely as sulphate, a large dose obscuring the sulphate action by the more energetic action of the sulphite. Animals differ very considerably in their oxidising powers; one will show sulphite action after two decigrams of sulphite, while another and apparently similar animal, will show very little sulphite action after one gram. The sulphate action consists of an intense metabolic stimulation, increased urine volume, urea, and other constituents. The sulphite action is a depressent action attacking the cell ferments and inhibiting them; this is shown by its action on metabolism, and also by its powerful inhibiting action on autolysis.

This action on the cell ferments appears to be somewhat complicated. The sulphite seems to combine with some part of the ferment, but apparently does not destroy it, for no compound containing sulphite can be found in the cells, while at the same time the cell ferments are

completely inhibited. If the sulphite is only sufficient to neutralise part of the ferment the animal recovers, oxidising ferments are brought in contact with the cells, and the sulphite is oxidised gradually to sulphate. Such an occurrence has been often observed in animals which have been apparently dead with sulphite poisoning, recovering completely.

The complete inhibition of the tissue ferments produces general symptoms usually described as central; that is, there is failure of respiration and also of circulatory centre, together with convulsions.

The sub-lethal action is as follows: There is produced an intense irritation of the renal epithelium which becomes more intense as the dose is increased; this is shown by the excretion of large quantities of casts, both epithelial and granular. There is also an interference with oxidation processes in the cells; this is shown by the presence of excess of the normal colouring matter of the urine and the presence of large quantities of calcium, and to a less extent magnesium salts, in the urine. These calcium salts are in the form of phosphate, carbonate and oxalate.

Sulphide. The sulphides are credited with having some action on suppurative processes; it was therefore sought to find out if there were any grounds for such an action.

As in the case of sulphates, hyposulphites and sulphites, all doses were given hypodermically. Great difficulty, however, was experienced owing to the extremely caustic nature of sodium sulphide. Kahlbaum's sodium sulphide was used for the first experiments, but Dr. Alexander Rule, Chemical Department, University of Liverpool, was kind enough to supply me with a quantity of sodium hydrogen sulphide of definite composition, the manufacture of which he had succeeded in discovering by precipitation in alcoholic solution. This preparation was found in every way more satisfactory, especially in its diminished causticity.

The first dose given was two centigrams of Kahlbaum's sodium sulphide, hypodermically. There was no urine passed during the following twenty-four hours, and this was followed next day by a small quantity of urine containing a diminished quantity of all the urinary constituents with the exception of sulphate. Casts were found in the urine, and also large quantities of insoluble calcium and magnesium salts, chiefly calcium carbonate and phosphate, with some calcium oxalate.

Injection of four centigrams of the sulphide was followed by two days' anuria; there was the following day a slight increase in the volume of urine and amount of urea and inorganic sulphates, but a

diminished excretion of organic sulphate. The insoluble calcium salts were present. Organic and inorganic sulphate was on this day as one is to thirty. The following day there was a large quantity of a substance which, when the urine was boiled with weak hydrochloric acid, smelled of creosote, and was probably combined with sulphate. The organic sulphate on this day had risen to one to five of inorganic. In all the following experiments the new sodium hydrogen sulphide of Rule was used.

Three decigrams of sodium hydrogen sulphide (0·3 gram) were given hypodermically to a rabbit. Within a short time its respirations became laboured and feeble; at the same time its heart beat became weak, and within three minutes it had a convulsion and died.

A fresh rabbit was given, hypodermically, five centigrams (0·05 gram). No urine was passed the following twenty-four hours; the following day, although the urine was less in quantity than normal, there was an increase in the urea and uric acid excreted; all the other constituents, including inorganic sulphates, were decreased; organic sulphates were slightly increased.

Amount	Urea	Inorganic sulphate	Organic sulphate	Ammonia	Uric acid
45	0·468	0·4500	0·045	0·0355	0·0310
Injection 0·05 gram sodium sulphide.					
No urine	—	—	—	—	—
40	0·900	0·166	0·048	0·0082	0·1216
42	0·496	0·290	0·068	0·0357	0·0464

A very large amount of insoluble salts were excreted on the third day. The sediment of these in bulk amounted to about one-third the urine volume. On shaking up, the consistency was extremely thick. These salts consisted chiefly of calcium carbonate, phosphates, chiefly calcium, and lesser quantity of calcium oxalate; there were also small quantities of magnesium salts. The carbonates in this urine were estimated, and represented four decigrams of calcium carbonate; there was nearly a gram of insoluble salts in this urine. Casts also were present in large quantity. A second rabbit reacted strongly to sodium sulphide, resembling the one previously given Kahlbaum's sulphide. One decigram of sulphide produced two days' anuria.

Another rabbit was given six centigrams of sodium sulphide hypodermically. There was an immediate diuresis closely resembling that seen after a dose of sulphate. Urine-volume, urea and other

constituents were roughly three times the normal amount. Total sulphates were very much increased, as also were organic sulphates. The sulphates were increased out of all proportion to the amount of sulphide given; nearly a gram and a half were excreted during the twenty-four hours. Casts, carbonates, phosphates and oxalates, chiefly of calcium, were present. A larger dose in the same rabbit of one decigram, hypodermically, gave a combination of the two effects. Normal amount of urine and its constituents, except slight increase in excretion of urea and excretion of insoluble calcium salts and casts, followed next day by the diuresis resembling sulphate diuresis. So that sulphide appears to resemble sulphite in its action in that it depends on the oxidation powers of the animal as to whether the injected compound should give the stimulating effects of sulphate, or the depressing effects of sulphide.

Experiments in Autolysis. These were performed in similar manner to those attempted in regard to sulphates and sulphites. Ten grams of fresh liver tissue was used in each case, toluol being used as a preservative. Very small quantities of sodium hydrogen sulphide were added. It was found, after incubating for seventy-two hours, that practically no autolysis had taken place in the ones containing sulphide, but, as with sulphites, tissues were found to differ very much in their resistance to this substance.

Lastly, it appeared of interest to find out whether sulphide formed a compound with any part of the living cells or whether it only became attached to the ferments. A rat was given two and a half decigrams of sodium hydrogen sulphide, hypodermically, and about half an hour afterwards was killed; the liver, spleen and kidneys were quickly removed, minced, ground in a mortar, and placed in a flask connected with another flask with glass tubing, the second flask containing a little copper sulphate. Two per cent. hydrochloric acid was added to the tissues, which were then heated. A distinct production of copper sulphide was discernible where the tube dipped into the copper sulphate, which would tend to show that sulphide forms a compound with some substance in the cells. A control rat was used, the organs in this case giving no sign of any sulphide.

We now attempted to further investigate the production of insoluble salts after an hypodermic injection of sodium hydrogen sulphide. One decigram was given hypodermically; the following day there was complete anuria.

The second day there was thirteen cubic centimetres of a dark urine, containing casts. The phosphates were the most prominent insoluble salts, somewhat less oxalates, and only a trace of carbonates.

The third day forty centimetres were passed. Phosphates were still in excess, carbonates had increased, oxalates decreased.

The fourth day thirty-five cubic centimetres; the oxalates had further decreased, carbonates increased, triple-phosphates increased.

The fifth day one hundred cubic centimetres of urine, sufficient to estimate the substances quantitatively. There was found to be present: calcium carbonate, 0.25 gram; calcium oxalate, 0.007 gram; calcium phosphate, 0.044 gram; ammonium and magnesium phosphates, 0.160 gram. The following day the urine was clear.

From these results it would appear that sodium sulphide, hypodermically, may have two separate actions. Firstly, in some animals possessing large quantities of oxidising ferments the sulphide may be almost immediately oxidised to sulphate, and then the action will be the pronounced diuretic action seen after injecting sulphate. Other animals having less oxidising ferments in their tissues will cause little or no oxidation of the sulphide, and the effects produced will then be the depressing effects due to the sulphide. Again, other animals will be found to be intermediate between these two, and there will be seen the mixed effect of the depressing sulphide and the stimulating sulphate.

If the dose of sulphide is lethal it combines with the cell tissues, or possibly the cell ferments, and produces cessation of all metabolic changes. The signs of such an action are paralysis of the respiratory and circulatory systems, convulsions, and death.

If the dose is sub-lethal, oxidase and other oxidising ferments attack the sulphide, which is then thrown off the cell in the form of sulphate, and this production of sulphate under such conditions is frequently in such minute amounts that no diuresis such as is seen after sulphate is to be found.

This sulphide combination in the cells produces a depressent action on tissue changes, but more especially on oxidation, so that unfinished products of oxidation are excreted. Calcium phosphate, carbonate and oxalate are invariably present in the urine, the carbonate being the most unusual and prominent, although the phosphates are found in larger amounts. In one day's urine four decigrams of calcium carbonate were found, and there were, besides, large quantities of phosphates and other salts. In another urine, on the fifth day after injection, two and a half

decigrams of calcium carbonate were found, together with about two decigrams of other insoluble salts.

Lastly, sulphides exert an extremely irritating effect on the renal epithelium, shown by the presence of casts in the urine after each hypodermic injection of the substance.

CONCLUSIONS

Sulphates, hyposulphites, sulphites and sulphides interfere with oxidation processes in the cells, sulphates in large amount by preventing exchange between the blood stream and the cells. This action disappears when the amount of sulphate is reduced, and there then follows a stage of stimulation, the sulphate causing a very marked diuresis, sometimes accompanied by the excretion of much more sulphates than were injected into the body together with the amount found in the food. Sulphate also has a marked irritant action on the kidney cells when more than a small amount is present. Purgation is not usual; during the second day after an injection an increased quantity of sulphates is excreted in the faeces, but the following day the amount has again fallen to normal. Calcium carbonate, phosphate and oxalate are found in the urine after large doses, and are probably products of incomplete oxidation, the oxidation having been interfered with by the high concentration in the blood preventing interchange with the cells.

Hyposulphites act very much like sulphates, being very quickly reduced in the body to sulphate.

Sulphites and sulphides are somewhat similar in their action, and this action depends on the amount of oxidising ferments found in the animal. In some animals they are at once oxidised, and the sulphates produced give the typical picture described above. If the animal is deficient in oxidising ferments the sulphite appears to unite with and neutralise the action of the tissue ferments. Sulphide appears to unite to some constituent of the cell itself, and both interfere, according to the quantity, with tissue changes, and if in sufficient amount they stop all metabolic changes and the animal dies of failure of respiration and circulation, with perhaps convulsions. If the amount is sub-lethal, it is attacked by the oxidising ferments, and gradually got rid of as sulphate. In the case of sulphite, when the toxic dose is large the sulphate gives the diuretic action, but in the case of sulphide the amount of sulphate is so minute that little or no diuresis is often to be seen.

Both sulphite and sulphide interfere with metabolic changes, especially with oxidation processes, causing the excretion of calcium especially, but also of other insoluble salts in the form of carbonate, phosphate and oxalate. There is also an intense renal irritation as the result of both these salts. The irritation caused by all these three sulphur compounds raises the possibility as to whether they may not, in part at least, be the cause of chronic nephritis and cirrhosis of the liver, both frequently ascribed to malted drinks, which are known to contain large amounts of sulphates. When it is also considered that these people usually have insoluble salts in their urine, such as are described in this paper, and as such results are not common after drinks which are free from sulphates, it is possible that the sulphates, and not the alcohol, may be the cause of their condition.

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