TABLE I. Production of Cardiac Necroses and Nephrocalcinosis by Stress in 8 Groups of Adrenalectomized Rats.

Treatment*	Cardiac necrosis (scale 0-3)	Nephrocalcinosis (scale 0-3)	Mortality (%)
F-COL	.0	.0	0
" + cold	1.3 ± .45	.0	10
" + motor denervation	2.7 ± .20	1.4 ± .30	70
" + restraint	2.3 ± .45	1.1 ± .30	90
Triamcinolone	.0	.0	0
" + cold	.0	.0	0
" + motor denervation	.0	.1 ± .10	30
" + restraint	.6 ± .30	.3 ± .20	0

* In addition to treatments listed in this column, all animals were adrenalectomized and given Na_2HPO_4 by stomach tube, as indicated in text.

Summary. In response to various stressors (cold, denervation, restraint), bilaterally adrenalectomized rats, suitably conditioned by pretreatment with certain corticoids and Na_2HPO_4, develop severe cardiac necroses and nephrocalcinosis. Apparently, these manifestations of stress are not mediated through increased secretion of adrenal hormones.

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Effect of Sodium Ferrocyanide and Bicarbonate on Reabsorption of Inorganic Sulfate in Renal Tubules.* (25730)

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Inorganic sulfate is reabsorbed by renal tubules in the dog by a mechanism exhibiting a transfer maximum (Tm)(7). Reabsorption is sensitive to a number of inorganic anions, such as chloride(2), phosphate and nitrate(6) and thiosulfate(1). This paper reports effects of sodium ferrocyanide and bicarbonate. The ferrocyanide ion was chosen because it is not reabsorbed in tubules in the dog(4). Bicarbonate was studied because of its marked effect on phosphate reabsorption (9).

Methods. Experiments were performed on unanesthetized female dogs in supine position. Suitable blood levels were obtained by intravenous priming injections and sustaining infusions. In each experiment, sulfate reabsorption was measured during 3-4 control periods, then in 3-5 periods during infusions of sodium ferrocyanide or bicarbonate. Filtered sulfate was always above Tm loads. In sodium bicarbonate experiments, arterial blood samples were collected anaerobically under paraffin oil. Venous blood samples were used in other experiments. Creatinine was analyzed by method of Bonsnes and Taussky(5), inorganic sulfate by method of Power and Wakefield (11) and bicarbonate according to Van Slyke and Neill(12). Ferrocyanide ion was determined colorimetrically as ferric ferrocyanide (Prussian blue) on trichloroacetic acid (10%) filtrates of plasma and urine, containing 0.1-0.3 μmoles Fe $(CN)_6^{-4}$/ml. 2 ml filtrate + 2 ml $Fe_2(SO_4)_3$-reagent (1 part 17% $Fe_2(SO_4)_3$ + 200 parts saturated solution of gum ghatti). After 20 minutes optical density was read at wavelength 660 mμ. In one ex-

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TABLE I. Effect of Sodium Ferrocyanide on Sulfate Reabsorption. Exp. 2. Dog: M (19 kg).

Time, min.	Urine flow, ml/min.	GFR, ml/min.	$Fe(CN)_6^{-4}$ plasma, μmoles/ml	Sulfate: Plasma, μmoles/ml	Sulfate: Urine, μmoles/min.	Sulfate: Reabsorbed, μmoles/100 ml GF
0	600 ml H_2O orally					
32	Prime 1: 4 g creatinine, 1.5 g Na_2SO_4. Start infusion 2.4 ml/min., 2.4% creatinine, 1% Na_2SO_4.					
79– 85	7.5	70		3.88	118	218
85– 91	8.1	71		3.80	123	206
91– 97	7.5	70		3.98	124	219
97–103	7.5	70		3.94	127	212
107	Prime 2: 7.5 g $Na_4Fe(CN)_6$. Start infusion 7.8 ml/min., 4% creatinine, 3% Na_2SO_4, 2.9% $Na_4Fe(CN)_6$.					
137–143	6.3	84	3.05	4.16	166	219
143–149	6.9	91	3.30	4.32	184	230
149–155	7.0	88	3.34	4.72	174	273
155–161	7.1	90	3.46	4.56	191	242

GFR = Glomerular filtration rate. GF = Glomerular filtrate.

periment, ferrocyanide was analyzed by method of Berliner *et al.*(4). Both sulfate and ferrocyanide precipitate with benzidine. Ferrocyanide was therefore removed by precipitation with *equivalent* amount of zinc acetate before analysis of sulfate. For plasma,

TABLE II. Effect of Sodium Ferrocyanide on Sulfate Reabsorption in 3 Experiments in Dogs.

Plasma $Fe(CN)_6$, μmoles/ml	Sulfate reabsorption: Control μmoles/100 ml GF	Sulfate reabsorption: $Fe(CN)_6$ μmoles/100 ml GF
4.1–4.9	164	155
5.2–6.2	159	139
3.0–3.5	214	241

0.01 M zinc acetate in 15% trichloroacetic acid was used, and an additional volume of the acid was added to yield a 3:1 plasma filtrate. Excess zinc must be avoided, since it facilitates precipitation of phosphate with benzidine, thereby giving too high sulfate values.

Results. Sodium ferrocyanide was given to 3 dogs in 3 experiments (Table I). Table II summarizes the 3 experiments with regard to reabsorption of sulfate before and after administration of sodium ferrocyanide, and ferrocyanide concentrations in plasma. There was no consistent change in reabsorption of sulfate.

Sodium bicarbonate was given to 4 dogs in 10 experiments. Table III shows a typical experiment. The 10 experiments are summarized in Table IV. Administration of sodium bicarbonate depressed reabsorption of sulfate in 9 experiments. The depression averaged 7%; it was not proportional to dose or

TABLE III. Effect of Sodium Bicarbonate on Sulfate Reabsorption. Exp. 15. Dog: E (23 kg).

Time, min.	Urine flow, ml/min.	GFR, ml/min.	Total CO_2 plasma, μmoles/ml	Sulfate: Plasma, μmoles/ml	Sulfate: Urine, μmoles/min.	Sulfate: Reabsorbed, μmoles/100 ml GF
0	700 ml H_2O orally					
12	Prime 1: 2 g creatinine, 1.5 g Na_2SO_4. Start infusion 3 ml/min., 1% creatinine, 8% Na_2SO_4.					
41–51	1.2	98	21.4	3.18	152	164
51–61	"	100	"	3.22	153	168
61–71	"	89	"	3.14	139	157
74	Prime 2: 2 g $NaHCO_3$. 3% $NaHCO_3$ added to infusion.					
80– 90	3.9	97	24.5	3.10	142	163
90–100	6.5	104	24.7	3.02	160	148
100–110	7.7	103	25.9	2.99	160	144
110–120	5.5	100	26.4	3.08	152	155
120–130	4.6	104	"	3.17	163	159

TABLE IV. Effect of Sodium Bicarbonate on Sulfate Reabsorption in 4 Dogs.

Total (CO_2)		Sulfate reabsorption		
Plasma, mmoles/l	Urine, mmoles/min.	Control μmoles/100 ml GF	$NaHCO_3$	% change
26–27	.03– .09	148	134	– 9
23–29	.01– .20	182	161	– 12
24–28	.03– .20	155	158	+ 2
24–26	.1 – .2	163	156	– 4
27–28	.3	173	151	– 13
22–29	.2 – .4	137	129	– 6
29–35	.3 – .7	194	191	– 2
29–36	.6 –1.2	118	112	– 5
35–43	.7 –1.7	148	138	– 7
37–40	1.3 –1.7	105	87	– 17
Avg control				– 7%
21–23	<.02			P <.005

plasma level of bicarbonate, and independent of time.

Discussion. According to their effect on reabsorption of sulfate, inorganic anions can be divided into 3 groups: 1. No effect or minor depression: ferrocyanide and bicarbonate ions; 2. Marked depression, immediate effect: thiosulfate(1), acetoacetate, nitrate and phosphate ions(6). 3. Marked depression, 30-50 minutes delay in onset: chloride (2).

Of the 2 ions in first group, ferrocyanide is neither reabsorbed nor secreted in kidneys of the dog, and probably never enters the tubule cells. Bicarbonate, in contrast, is reabsorbed by the tubules. Reabsorption of bicarbonate in distal tubules involves exchange of hydrogen ions for sodium ions(10). The bicarbonate in urine is then converted to carbonic acid, and CO_2 and water diffuse back across the tubular epithelium. The same mechanism probably accounts for reabsorption of bicarbonate in proximal tubules(3). It is therefore possible that no bicarbonate ions enter tubule cells from urine.

The minute effect of bicarbonate on sulfate reabsorption is of doubtful physiological significance, and cannot compare with the effect on phosphate reabsorption (30% depression) (9), which might be due to change in ratio between $H_2PO_4^-$ and $HPO_4^=$ (pK^2 of phosphoric acid = 7.12) in tubular urine, when pH is raised(8).

All anions which depress reabsorption of sulfate (Groups 2 and 3) are themselves reabsorbed in renal tubules. Maximum reabsorption rates of nitrate, acetoacetate or chloride are more than 3 times as high as sulfate Tm(6), but phosphate(6) and thiosulfate(1) have lower Tm than sulfate. Evidently the depressing action of these ions is not merely related to their turn-over rate in tubule cells, but also to their affinity to sulfate transport system.

Summary. Sodium ferrocyanide and sodium bicarbonate have almost no effect on reabsorption of sulfate in renal tubules in the dog. These results, together with earlier studies, suggest that only anions which are reabsorbed as such through tubule cells, depress reabsorption of sulfate. This assumes, however, that bicarbonate is reabsorbed only as CO_2 and water, and not in ionic form.

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