A STUDY OF SULFUR METABOLISM AND THE EFFECT OF SULFUR ADMINISTRATION IN CHRONIC ARTHRITIS [1]

By R. H. FREYBERG, W. D. BLOCK, and M. F. FROMER

(From the Rackham Arthritis Research Unit, the Medical School, University of Michigan, Ann Arbor) [2]

(Received for publication November 21, 1939)

There are conflicting opinions concerning the use of sulfur in the treatment of arthritis. Most of the reports deal with *clinical* observations and on this basis some authors (1) enthusiastically recommend the use of sulfur, others (2) condemn it as valueless.

Arthritis is one of many diseases that for centuries has been treated empirically with sulfur administered orally, by inunction, or in mineral water baths. Approximately fifty years ago sulfur was first prepared in colloidal suspension by Debus (3), and soon thereafter this type of preparation became the most popular form of sulfur medication. Why this is true we do not know. We have been unable to find any scientific reason why *elemental sulfur in colloidal suspension* has been used in the treatment of arthritis or why it has been administered *parenterally* (intramuscularly or intravenously).

In the literature recommending sulfur therapy, one encounters statements such as: "In arthritis, there is a marked disturbance of sulfur metabolism"; "A deficiency of sulfur in the tissues of arthritics has been reported"; "The patient with rheumatoid arthritis has lost the capacity for retaining sulfur in the tissues." Other less specific statements include "sulfur tends to normalize body metabolism and vitalize cell tissues by supplying sulfur deficiency . . ."; "There seems to come a definite feeling that the arthritic patient is one who has a low sulfur reserve," etc. Actually, however, only a few investigations of sulfur metabolism in patients with arthritis have been made.

The first sulfur balance observations on arthritic patients known to us are those of Goldthwait, Painter and Osgood (4). Three patients were studied; one with hypertrophic arthritis retained 60 per cent of the sulfur ingested during an eight-day period, and two patients with atrophic arthritis eliminated more sulfur than they ingested. An extensive review of the literature concerning colloidal sulfur in the treatment of arthritis has recently been published (5), to which the reader is referred for a discussion of the work of Meyer-Bisch, Cawadias and Race which deals with certain considerations of sulfur metabolism.

Senturia (6) studied the urinary sulfur in patients with rheumatoid arthritis, hypertrophic arthritis and healthy individuals while they followed their "customary dietary regime." The average values for the different fractions of sulfur and the N/S ratio for the three groups were essentially the same. Senturia concluded that "the sulfur excretion and sulfur partition in the twenty-four-hour quantities of urine of eighteen patients with atrophic arthritis and forty-one with hypertrophic arthritis showed no appreciable deviation from those of twenty healthy individuals. . . . Our experiments disprove the alleged existence of an abnormal sulfur elimination or sulfur partition in the urine of patients with chronic non-specific arthritis.

In an effort to learn how sulfur might be helpful in arthritis, Wheeldon (7) recently analyzed "twenty-four-hour collections" of urine for total sulfur and its partitions. He found an average reduction in sulfate sulfur excretion in thirty-four arthritics; the average neutral sulfur and ethereal sulfate excretion, however, were essentially normal. No difference was found between patients with hypertrophic and proliferative arthritis in regard to the excretion of total sulfur and neutral sulfur. His subjects were out-patients and it is admitted that "some of the specimens (of urine) undoubtedly did not represent true twenty-four-hour specimens." The urine volumes varied greatly; no mention is made of creatinine determinations, and the analytical results were com-

[1] Read, in part, before the Sixth Annual Meeting of the American Rheumatism Association, Saint Louis, Missouri, May 15, 1939.

[2] The Rackham Arthritis Research Unit is supported by the Horace H. Rackham School of Graduate Studies of the University of Michigan.

pared with normal values of other investigations. Consequently, it is difficult to evaluate these results. Analysis of blood from twenty-five arthritic patients showed normal total sulfur, total sulfate and average inorganic sulfate content and no variation in the average reduced glutathione content following administration of sulfur.

In the small amount of literature dealing with sulfur metabolism in relation to arthritis, there is much that is contradictory and the data certainly do not support the numerous far-reaching and diverse statements that are made concerning altered sulfur metabolism, sulfur deficiency, and the like, in patients with arthritis.

The rationale for the administration of sulfur to patients with arthritis is based primarily on the following claims: (*1*) It is said that a deficiency of sulfur exists in chronic arthritics, as evidenced chiefly by a lowered content of cystine in fingernails (8), and by a reduced sulfur content of articular cartilage (9). (*2*) It is reported that free indole is often found in the urine of arthritics and, since oxidized sulfur is used in the detoxification of indole, it is *inferred* that an insufficient supply of sulfur exists for the complete detoxification of this substance or that, due to some metabolic fault, available sulfur is not used normally for this purpose.

Because of the disagreement concerning the value of sulfur therapy and because there is insufficient knowledge of the metabolism of sulfur in arthritics, the investigations herein reported were conducted. The studies were planned primarily to answer two questions: (*1*) Is there a deficiency of sulfur or an abnormality of sulfur metabolism in patients with chronic arthritis? (*2*) If there is such a deficiency or abnormality, does the administration of sulfur in various forms benefit or correct either? No attempt was made to evaluate sulfur therapy on the basis of *clinical* changes in the patients studied.

PROCEDURE

Subjects

The metabolism studies were made on patients with various types of arthritis and on normal individuals (see Table I). Four patients had rheumatoid arthritis in different stages of the disease: Three were males, one was a female; their ages ranged from twenty-six to forty years. The disease had existed only three months in O. J. at the time the investigation began. Many of the affected joints were acutely inflamed and fever of one to two degrees occurred daily throughout the period of study. G. Mc. was in the subacute stage of the disease. Several joints showed signs of active inflammation, in others the inflammation had subsided; at times he had slight fever. S. K., who was in the early chronic stage of the disease, had had arthritis for twenty months when these studies began. All involved joints showed considerable periarticular swelling; in some joints inflammation was slightly active, while in others only the residual changes of previous inflammation existed, especially contractures and impairment of motion due to cartilage destruction. C. B. was incapacitated because of arthritis which he had had for six years. There were no clinical signs of active joint inflammation but the erythrocyte sedimentation rate was elevated. His chief difficulties were marked limitation of joint motion, multiple flexion contractures and pain in the weight-bearing joints when standing. He represented the late chronic stage of the disease when joint damage from previous inflammation was of paramount importance.

The three patients with spondylitis rhizomelica varied in age from eighteen to thirty-one years; all were males. R. H., who was in the early stage of spondylitis, had symptoms only in the low dorsal and lumbar spine and sacro-iliac joints. Pain was the outstanding symptom; limitation of motion was only moderate and due chiefly to muscle spasm and pain. S. G. had had symptoms for six years. There were pain and immobility of the dorsolumbar spine. Roentgenograms showed obliteration of sacro-iliac joints and beginning calcification of longitudinal spinal ligaments. J. S. had had spondylitis for twelve years; he no longer had pain but was almost completely incapacitated because of rigidity of the entire spine in severe kyphosis and ankylosis of both hips. Extensive calcification of the ligaments was demonstrated by roentgenograms.

Two patients with typical degenerative disease of the joints ("hypertrophic," "osteo" arthritis) were studied. One, D. M., a female, sixty-one years, had typical bilateral malum coxae senilis and no other clinical arthritis. The other, G. M., a male, sixty-one years, had typical clinical and roentgenological changes in many of the distal phalangeal finger joints, a few proximal phalangeal finger joints and in both knees. The disease had existed for ten and six years, respectively, in these patients.

These differences in sex, age and clinical characteristics of the disease clearly show that these patients represent all of the important stages of each of the types of arthritis studied. None of the arthritics had any other disease which in any known way would affect the sulfur metabolism.

The five control subjects were healthy persons varying in age from twenty-three to fifty-one years. One was a female. D. Mc. was an unemployed laborer; the others were students.

TABLE I

Average twenty-four hourly urinary excretion of nitrogen and sulfur in all subjects during control periods

Subject	Sex	Age	Body weight	Duration of illness	Nitrogen excretion	Urinary sulfur excretion: Total sulfur	Total sulfate sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur		N/S ratio
			pounds	*months*	*grams*	*mgm.*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	
Normals:															
W. A.	M	27	165		9.16	757	622	(82)	567	(75)	55	(7)	135	(18)	12.04
D. Mc.	M	51	159		8.90	711	594	(84)	548	(77)	46	(6)	116	(16)	12.52
H. M.	M	25	111½		7.42	640	505	(79)	473	(74)	33	(5)	135	(21)	11.60
M. H.	F	28	140		7.53	621	490	(79)	458	(74)	32	(5)	131	(21)	12.12
E. W.	M	23	179		8.26	680	554	(81)	522	(76)	28	(4)	130	(20)	12.13
Average			151		8.25	682	553	(81)	518	(75)	39	(5)	129	(19)	12.08
Rheumatoid arthritics:															
G. Mc.	M	26	154	6	9.01	695	558	(80)	490	(70)	69	(10)	136	(20)	12.96
O. J.	M	49	146	3	8.98	667	552	(83)	490	(74)	62	(9)	114	(17)	13.46
S. K.	F	26	110½	20	7.29	540	478	(88)	424	(78)	52	(10)	64	(12)	13.50
C. B.	M	50	144½	72	8.14	698	540	(77)	476	(68)	62	(9)	159	(23)	13.10
Average			139		8.35	650	532	(82)	470	(73)	61	(9)	118	(18)	13.09
Hypertrophic arthritics:															
G. M.	M	61	150	72	8.12	661	524	(79)	476	(72)	48	(7)	137	(21)	12.28
D. M.	F	61	125	120	7.37	623	526	(84)	442	(71)	84	(12)	96	(15)	11.83
Average			137		7.75	642	525	(82)	459	(72)	66	(10)	117	(18)	12.06
Spondylitis rhizomelicas:															
R. H.	M	18	110	36	6.23	520	411	(80)	363	(70)	48	(9)	108	(20)	11.98
S. G.	M	30	148	96	8.28	621	516	(83)	460	(74)	56	(9)	105	(17)	13.33
J. S.	M	31	130	144	7.70	599	501	(83)	451	(75)	50	(8)	98	(16)	12.85
Average			129		7.40	580	476	(82)	425	(73)	51	(9)	104	(18)	12.72

Plan of study

All subjects were fed identical diets except for the addition of butter, cream, sugar or mayonnaise to some in order to provide sufficient energy to prevent weight loss in larger and more active subjects. This was arranged so that the sulfur intake would be the same in all cases. Three menus were arranged for successive days; these were rotated in order throughout the study. The protein content of the diet was kept low, 60 grams, so that the intake of sulfur (source of which is chiefly protein) would be relatively small. In this way the basal excretion of sulfur would be small and changes in sulfur elimination during periods of sulfur therapy would be more sharply contrasted. The dietary protein was sufficiently high so that all subjects were in positive nitrogen balance.

The meals for all subjects were quantitatively prepared in the special metabolism kitchen and were served to the patients on the wards where they resided throughout the period of study. Control subjects lived outside of the hospital and ate all their meals in the metabolism dining room. Each subject drank a fixed amount of distilled water daily. All of the food was eaten each day and vomiting or diarrhea did not occur.

After four to seven days were allowed for adjustment to the diet, all urine was saved in twenty-four hourly collections and preserved with toluene. Urine obtained in this way was analyzed for total nitrogen, total sulfur, total sulfate sulfur, inorganic sulfate sulfur, and by difference the conjugated sulfate sulfur and organic sulfur were calculated. Complete collection of urine was assured by daily creatinine analyses.

After a period of study (four to twelve days) when the diet provided the sole intake of sulfur, various experiments were conducted. Sulfur was administered in different forms and by different routes, but similarly to patients and controls, and changes in urinary sulfur were measured. Thymol was fed to some of the subjects to test the ability to conjugate inorganic sulfur. Experimental periods were always separated by many days of "control" when no medication was given. A terminal control period ended the investigation. The average daily urinary sulfur excretion during experimental periods was contrasted with the average daily excretion during control periods.

Finger-nail clippings were analyzed for cystine before the administration of sulfur and at approximately monthly intervals thereafter for three to seven months. The urine of eight of the arthritics and three of the controls was analyzed for indole daily.

Chemical methods

Urinary sulfur was partitioned according to the technique of Folin (10). Total sulfur was determined by the method of Denis-Benedict (11) and total nitrogen by the usual macro-Kjeldahl procedure. Cystine was determined by the Sullivan method as modified by Rossouw and Wilken-Jorden (12), creatinine by the method of Folin (13) and indole by the procedure of Forbes and Neale (14) controlled by analysis of pure indole and a water blank to insure against false positive results.

EXPERIMENTAL DATA

Sulfur is eliminated almost entirely in the urine. A relatively small and fixed amount passes through the skin (15) and none is excreted by the bowel except as hydrogen sulfide which is formed by bacteria in the intestinal tract. Thus sulfur metabolism can be accurately studied by a complete analysis of urine; the small amount of sulfur eliminated by other routes was not measured.

In order to compare the sulfur and nitrogen metabolism of patients with arthritis and normal individuals, the average daily excretion of nitrogen and urinary sulfur distribution during control periods was determined. The number of control days varied in different subjects from fourteen to twenty-five. These data appear in Table I.

It should be noted that the total elimination of sulfur is essentially the same in the controls and all arthritics, although it is slightly higher in the controls as would be expected because of their larger size and greater activity. The per cent of sulfur eliminated as total sulfate, as inorganic sulfate and as organic sulfur is practically identical in all groups. The conjugated sulfate sulfur averages 9 or 10 per cent in different groups of arthritics and 5 per cent in the control subjects. It is this conjugated sulfate fraction which indicates the amount of sulfur combined with phenolic substances such as indole. The differences in the excretion of conjugated sulfate sulfur shown in Table I may be of no importance since the amount of sulfur eliminated as conjugated sulfate in *normal* individuals has been found to vary from 3 to 15 per cent of the total urinary sulfur. If these differences are significant, they certainly indicate no deficiency in the available supply of sulfur and no impairment in the use of sulfur for this means of detoxification by the arthritic patient. The average N/S ratio varies only slightly in the different groups of subjects; we can attribute no significance to this slight variation.

From the data presented in Table I the following significant conclusions are drawn:

No important difference in the urinary sulfur distribution of patients with rheumatoid arthritis, hypertrophic arthritis, spondylitis rhizomelica, and normal individuals exists unless it be the slightly higher per cent of sulfur eliminated as conjugated sulfate by the arthritics. If this difference is significant, it certainly indicates *no deficiency in sulfur available for detoxification, and no impairment in this detoxifying function in arthritics.*

The effect of colloidal sulfur injected intravenously

To study the effect of the intravenous and intramuscular injection of colloidal sulfur, a preparation called "Sulisocol"[3] was used. It was prepared so that one cubic centimeter contained 10 mgm. of sulfur. Our analyses of this preparation showed that it contained no nitrogen and no organic material. This preparation was selected in order that we might study the effect of injection of sulfur alone, without simultaneously injecting protein or any other substance which might affect the sulfur metabolism. In all subjects, the colloidal sulfur was injected intravenously over a period of three days as follows: On the first day, 20 mgm. were injected in the morning, 20 mgm. in the evening; on the second and third days 50 mgm. were injected both morning and evening. Thus, according to the statement of content on the medicament 250 mgm. of sulfur were injected during the three-day period. (By our analysis it was found that we actually injected 260 mgm. of sulfur.) In control subject E. W. one 50 mgm. injection was omitted, and he was given only 208 mgm. during the three-day period. The medicine was always injected slowly (at the rate of 1 cc. per minute). Four controls and all the arthritis subjects were studied in this way. The experiment was carried out twice on the patients with

[3] Kindly supplied for this study by The Drug Products Company, Long Island City, New York.

rheumatoid arthritis. Fever did not occur after any injections and no undesirable effect was observed.

The large amount of sulfur injected when the dietary source of sulfur was low produced a definite increase in excretion of sulfur and was completed in the majority of cases within twenty-four hours after the last day of injection. The results of intravenous injections of colloidal sulfur appear in Table II. In this and similar tables to follow the effect of the administration of sulfur is measured by determining the amount of total sulfur and of each sulfur fraction excreted in the urine *in excess of the average excretion* during the control periods. If less sulfur was eliminated during the experimental period than the average during the control periods, the value appears as a negative quantity.

Examination of Table II shows that in normal subjects the sulfur excretion increased from 76 to 135 per cent of the amount of sulfur injection.

TABLE II

The effect of colloidal sulfur injected intravenously

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods							
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur	
	mgm.	*mgm.*	*per cent of added sulfur*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*
Normals:									
W. A.	260	198	(76)	159	(80)	− 6	(−4)	48	(24)
D. Mc.	260	244	(94)	128	(52)	−36	(−15)	144	(64)
H. M.	260	352	(135)	324	(92)	− 4	(−1)	28	(8)
E. W.	208	237	(114)	153	(64)	30	(12)	54	(24)
Average			(105)		(72)		(−2)		(30)
Rheumatoid arthritics:									
G. Mc.	260	308	(165)	224	(72)	− 4	(−3)	84	(36)
	260	488	(188)	308	(63)	12	(4)	164	(34)
O. J.	260	304	(117)	256	(84)	4	(1)	48	(15)
	260	476	(183)	336	(71)	0	(0)	140	(29)
S. K.	260	196	(75)	204	(104)	12	(6)	−24	(−12)
	260	200	(77)	216	(108)	−20	(−10)	8	(4)
C. B.	260	520	(200)	300	(58)	−64	(−13)	288	(55)
	260	324	(125)	320	(99)	−64	(−21)	72	(22)
Average	260	352	(141)	270	(82)	−15	(−4.5)	98	(23)
Hypertrophic arthritics:									
G. M.	260	300	(115)	268	(90)	−28	(−9)	56	(19)
D. M.	260	332	(128)	316	(95)	4	(2)	4	(2)
Average	260	316	(122)	292	(93)	−12	(−4)	30	(11)
Spondylitis rhizomelicas:									
S. G.	260	285	(110)	225	(79)	18	(5)	45	(16)
J. S.	260	496	(191)	288	(58)	132	(27)	76	(15)
	260	424	(163)	240	(57)	52	(12)	128	(31)
Average	260	401	(155)	248	(65)	67	(15)	82	(21)

The arthritics eliminated from 75 to 200 per cent of the injected sulfur. In all but one arthritic (S. K.) the total sulfur excretion increased by amounts *much greater than the amount of sulfur injected.* The excess sulfur was eliminated chiefly as inorganic sulfate in both the controls and arthritics. The conjugated sulfur which, it must be remembered, is the form in which sulfur is eliminated when it is conjugated with phenolic substances, changed insignificantly or was actually *less* than during control periods *in all arthritics except one* with spondylitis (J. S.). No important increase in elimination of conjugated sulfur occurred in any of the patients with rheumatoid or hypertrophic arthritis. In only three of the ten observations made on these patients was there any noticeable increase in conjugated sulfur excretion and in each of these instances the increase was much less than that occurring in one control subject. The average change in conjugated sulfur excretion in these groups of arthritics was *negative.* In only one of the three observations made on the two subjects with spondylitis, and thus in only one of a total of thirteen observations on arthritics, was there a greater increase in excretion of conjugated sulfate than occurred in the control subjects. It did not occur again when the patient was studied later.

The organic sulfur excretion did not change consistently. There was no difference between the arthritics and control subjects in this sulfur fraction. (It should be noted that through the entire study greatest changes in organic sulfur excretion occurred when the excretion of conjugated sulfur changed in the opposite direction.)

The following conclusions concerning the effect of intravenous injection of colloidal sulfur are drawn: *The effect of colloidal sulfur injected intravenously is essentially the same in arthritics and in controls. Since the sulfur excretion increased by amounts greater than the amount injected, certainly the intravenous administration of colloidal sulfur would not benefit or prevent a sulfur deficiency if one existed. Since no significant increase in conjugated sulfur excretion occurred except in one of the thirteen observations made on eight arthritics, detoxification cannot be an important benefit resulting from the injection of colloidal sulfur.*

The effect of colloidal sulfur injected intramuscularly

Intramuscular injections of the same preparation of colloidal sulfur were given in the same amounts and according to the same schedule as in the intravenous studies. The results were observed in a similar way and appear in Table III. By our analysis it was found that we injected 270 mgm. of sulfur in the three-day period instead of 250 mgm., which was the amount injected according to the statement of content on the medication. In two subjects W. A. (control) and D. M. (hypertrophic arthritis) the medicine caused moderate pain at the site of injection and, consequently, injections were stopped after 162 mgm. had been given. No undesirable effects were noted; fever did not occur. Intramuscular injections were given to three controls and to the same arthritics that were formerly studied with intravenous injection of sulfur.

Examination of Table III shows that *all* subjects, arthritics and controls, had increased excretion of sulfur during the period of intramuscular injections; in fact, an excretion even greater than the amount of sulfur injected. Here again, the excess sulfur excretion was chiefly inorganic sulfate, as was the case when colloidal sulfur was given intravenously. In order to determine whether muscle destruction might account for this increased sulfur excretion, a non-sulfur-containing fluid was injected intramuscularly in one of the control subjects. Five cubic centimeters of calcium levulinate were injected on one day and ten cubic centimeters on the following day. No change in the excretion of nitrogen, total sulfur or any of its fractions occurred, indicating that muscle catabolism was not responsible for the increased sulfur excretion.

TABLE III

The effect of colloidal sulfur injected intramuscularly

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods							
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur	
	mgm.	*mgm.*	*per cent of added sulfur*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*
Normals:									
W. A.	162	460	(284)	320	(70)	−28	(−6)	168	(36)
D. Mc.	270	294	(109)	192	(65)	36	(12)	60	(23)
E. W.	270	330	(122)	400	(121)	35	(10)	−105	(−34)
Average			(171)		(85)		(8)		(13)
Rheumatoid arthritics:									
G. Mc.	270	480	(178)	355	(74)	−75	(−16)	200	(42)
O. J.	270	628	(233)	432	(69)	32	(5)	164	(26)
S. K.	270	512	(190)	480	(94)	36	(7)	−8	(−1)
C. B.	270	396	(147)	220	(55)	68	(17)	112	(28)
Average	270	504	(187)	122	(73)	15	(3)	117	(24)
Hypertrophic arthritics:									
G. M.	270	305	(115)	260	(89)	− 5	(−4)	45	(15)
D. M.	162	246	(152)	150	(61)	−15	(−7)	114	(46)
Average			(133)		(75)		(−5)		(30)
Spondylitis rhizomelicas:									
S. G.	270	345	(128)	245	(71)	−40	(−12)	140	(41)
J. S.	270	435	(161)	285	(66)	−55	(−13)	205	(49)
Average	270	390	(145)	265	(68)	−43	(−12)	173	(45)

When colloidal sulfur was injected intramuscularly the excretion of conjugated sulfate varied slightly in different subjects, but in only one of the eight arthritics studied was this sulfur fraction increased by an amount greater than was observed in normal control subjects. This same patient showed the greatest *negative* change in the excretion of conjugated sulfate when colloidal sulfur was injected intravenously (see Table II). At no time during this study did this patient have indoluria. It should be noted further in this regard that (J. S.) the patient with spondylitis, who was the only arthritic to excrete more conjugated sulfate than controls when colloidal sulfur was given intravenously (see Table II), had one of the greatest *negative* changes in this fraction of urinary sulfur during the intramuscular studies. The differences in conjugated sulfate excretion in the case of each of these arthritics to whom attention has been directed are unimportant. No consistent change in the excretion of organic sulfur occurred.

From this study we conclude: *Without exception the excretion of sulfur increased by amounts greater than the amount of sulfur injected intramuscularly, thus tending to create rather than prevent a deficiency of sulfur. With the possible exception of one patient, no important change in the excretion of conjugated sulfate occurred.*

The effect of colloidal sulfur given orally

Two normal subjects and the four patients with rheumatoid arthritis were given Mulford's "sulphocol," a preparation of colloidal sulfur designed for oral use. Each subject ingested six capsules in three equal doses during one day, providing 276 mgm. of sulfur (our analysis). The results appear in Table IV.

TABLE IV

The effect of colloidal sulfur orally

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods							
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur	
	mgm.	*mgm.*	*per cent of added sulfur*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*
Normals:									
W. A.	276	247	(89)	220	(89)	14	(5.7)	13	(5.3)
D. Mc.	276	378	(137)	296	(76)	16	(6)	62	(18)
Average	276	312	(113)	258	(82)	15	(6)	37	(12)
Rheumatoid arthritics:									
G. Mc.	276	278	(101)	208	(75)	18	(7)	52	(19)
O. J.	276	224	(85)	235	(105)	2	(1)	−12	(−6)
S. K.	276	228	(83)	224	(98)	6	(2)	− 1	(0)
C. B.	276	157	(57)	173	(110)	−13	(−8)	− 2	(−2)
Average	276	222	(81)	210	(97)	3	(0.5)	9	(3)

The sulfur excretion changed in essentially the same way in the arthritics and the controls. The total sulfur excretion increased by amounts nearly equal to the amount given. When the excess excretion was less than the sulfur medication, the difference can reasonably be accounted for by incomplete absorption and by elimination of sulfur by the bowel in the form of hydrogen sulfide. The added sulfur was eliminated chiefly as inorganic sulfate. In none of the subjects was there a significant increase in the excretion of conjugated sulfate or organic sulfur. The medication caused no discomfort. Its effect was over inside of twenty-four hours after administration.

From this study the following conclusions are drawn: *Colloidal sulfur can be safely given orally. When so administered it is quickly eliminated almost entirely as inorganic sulfate; it is not used for conjugation and excreted as ethereal sulfate. Colloidal sulfur is metabolized and excreted in the same way whether given intravenously, intramuscularly or orally, thus indicating no advantage in parenteral use of the drug.*

The effect of sodium thiosulfate given orally

Since elemental colloidal sulfur is eliminated in the urine chiefly as oxidized sulfur, we wished to determine the effect of an inorganic sulfur-containing compound given orally. Sodium thiosulfate was used because relatively large amounts of this salt can be given without producing diarrhea.

The same subjects on whom the effect of oral colloidal sulfur was studied, and also one patient with spondylitis rhizomelica and two patients with hypertrophic arthritis were given sodium thiosulfate. In every case two grams of the salt were given in capsules in one dose after breakfast. The sulfur intake was thus increased by 810 mgm.

Table V shows the results are similar in arthritics and controls. The excess urinary sulfur excretion was less than the amount given. At first it might be thought that this represents retention of sulfur; however, the fact that the control subjects showed precisely the same results as *all* of the arthritics would indicate that this is not the case. The difference can very reasonably be accounted for by the elimination as hydrogen sulfide gas by the bowel; also some of the salt may not have been absorbed. As in the case of previous studies, the excess sulfur in the urine was excreted almost entirely as inorganic sulfate; no significant change occurred in conjugated sulfate excretion. The effect was completed inside of twenty-four hours after ingestion of the salt.

This study shows that: *Sulfur given orally as sodium thiosulfate is quickly excreted almost entirely as inorganic sulfate by arthritics and controls and none is used for detoxification.*

TABLE V

The effect of 2 grams of sodium thiosulfate orally

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods							
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur	
	mgm.	*mgm.*	*per cent of added sulfur*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of tota*
Normals:									
W. A.	810	438	(54)	414	(95)	12	(2.7)	10	(2.3)
D. Mc.	810	578	(71)	524	(91)	−6	(−1)	56	(11)
Average	810	508	(62)	469	(93)	3	(1)	33	(7)
Rheumatoid arthritics:									
G. Mc.	810	596	(74)	554	(93)	4	(1)	36	(6)
O. J.	810	364	(45)	368	(101)	− 7	(−2)	4	(1)
S. K.	810	460	(57)	441	(96)	1	(0)	19	(4)
C. B.	810	484	(54)	524	(108)	−26	(−5)	−14	(−3)
Average	810	476	(58)	472	(99)	− 4	(−1.5)	11	(2)
Hypertrophic arthritics:									
G. M.	810	356	(44)	314	(89)	23	(7)	19	(6)
D. M.	810	466	(58)	466	(100)	−14	(−3)	15	(3)
Average	810	411	(51)	390	(95)	5	(2)	17	(5)
Spondylitis rhizomelica:									
S. G.	810	536	(66)	502	(94)	−15	(−3)	49	(9)

The effect of thymol administered orally

Even though the excretion of sulfur during control periods indicated no impairment of detoxification by conjugation of potential toxins with sulfur, and though sulfur administered in different ways was not used for this purpose, we wished to study this detoxifying mechanism further because of the prevalent belief that there may be a fault in the metabolism of sulfur which interferes with sulfur conjugation. Figure 1 indicates the manner in which phenolic substances are combined with sulfuric acid and excreted. Indole is regarded by some investigators (16) as an important factor in the causation of arthritis. We wished to determine the ability of arthritics to eliminate a phenolic substance quantitatively administered. We chose thymol as the test chemical because it is non-toxic and can be safely given orally in amounts sufficient to make an adequate test (17).

One-half a gram of thymol was given in capsules to three control subjects and to all of the arthritic patients studied. This medication was given in one dose after breakfast. If all the thymol were eliminated as ethereal sulfate, 107 mgm. of sulfur would be required for the conjugation. If impairment of this detoxifying mechanism existed in any of the arthritics, the increase in excretion of conjugated sulfate during the period of thymol administration would be significantly less than that of the control subjects studied in the same way.

The results of this investigation are shown in Table VI. As would be expected, the total sulfur excretion was not importantly affected. The excretion of conjugated sulfate was significantly increased in all subjects and the inorganic sulfate elimination was very definitely decreased in all subjects. Thus, it appears that thymol was conjugated with sulfur that otherwise would have been eliminated chiefly as inorganic sulfate. No consistent change in organic sulfur was noted. It was disappointing to us that this test was so indelicate. The amount of conjugated sulfate excreted varied considerably in the different subjects. The widest range was noted in the control group. In each group of arthritics the average conjugated sulfate excretion was increased slightly less than it was in the control group. This is due to the one high value in control W. A. which was 125 per cent of the theoretical total. It is obviously impossible to have more than 100 per cent conjugation of thymol. This high value in W. A. is undoubtedly due to a *basal excretion* of conjugated sulfate considerably greater than the average on the experimental day when a high percentage of thymol was also conjugated. It is unfortunate that this occurred for it falsely suggests a difference in the two groups which undoubtedly does not exist. It is most important to note that the

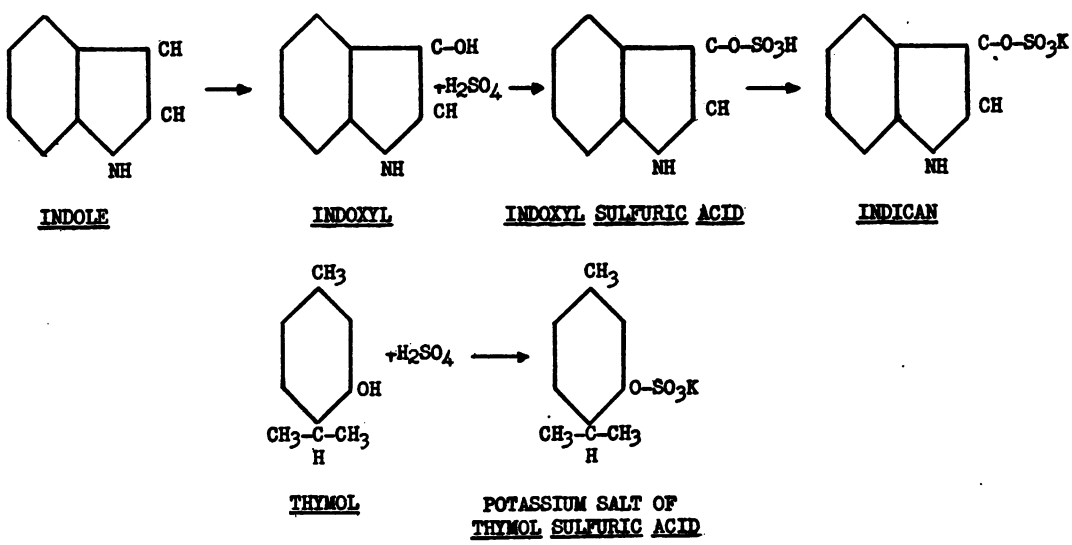

FIG. 1. SHOWING THE MANNER IN WHICH PHENOLIC COMPOUNDS ARE CONJUGATED WITH SULFURIC ACID

TABLE VI

Effect of the ingestion of 0.5 gram of thymol

Subject	Sulfur excretion compared to average during control periods				
	Total sulfur	Inorganic sulfate sulfur	Conjugated sulfate sulfur		Organic sulfur
	mgm.	*mgm.*	*mgm.*	*per cent of theoretical total**	*mgm.*
Normals:					
W. A.	−64	−80	140	(125)	−124
D. Mc.	− 1	−48	66	(62)	− 21
E. W.	0	−83	40	(43)	30
Average	−21	−70	82	(77)	− 38
Rheumatoid arthritics:					
G. Mc.	−16	− 86	103	(96)	−33
O. J.	−29	−131	55	(51)	48
S. K.	− 2	− 66	51	(48)	14
C. B.	− 3	−112	50	(47)	60
Average	−12	− 99	65	(61)	22
Hypertrophic arthritics:					
G. M.	−65	−125	49	(45)	11
D. M.	70	− 14	58	(54)	26
Average	3	− 55	54	(49)	18
Spondylitis rhizomelicas:					
S. G.	−25	−92	43	(40)	24
J. S.	67	−65	95	(89)	37
Average	21	−78	69	(64)	30

*0.5 gram thymol requires 107 mgm. sulfur for complete conjugation.

excretion of conjugated sulfate by all arthritics was within the range of excretion of this fraction of sulfur in the control subjects.

This study leads to the conclusion that: *No deficiency of sulfur available for conjugation with phenolic substances exists in arthritics, and no impairment of this detoxifying mechanism is evident in arthritics.*

In this connection it is interesting to note that the urine collected from six of the eight arthritics and from two of the controls was tested daily for indole, and the results were entirely negative each day on all subjects but one, J. S., a patient with spondylitis, who had slight traces of indole in the urine on only two successive days. It is interesting that this indoluria occurred after the patient had received large amounts of sulfur, and actually during a test period when colloidal sulfur was being injected intravenously.

Finger-nail cystine studies

The cystine content of finger-nails of all subjects was determined before any sulfur preparations were administered and at approximately monthly intervals thereafter, usually for three or more months. The data appear in Table VII. To better evaluate the results obtained in these subjects, analyses of finger-nails of other persons were made and are tabulated in Table VIII.

Normal individuals are reported by Sullivan and Hess (8) to have finger-nail cystine of from 10.28 to 13.02 per cent; the average value was 11.69 per cent. The cystine values for normal finger-nails reported by Klauder and Brown (18) are: range, 10.9 to 13.5 per cent, average 12.0 per cent. By a modification of Sullivan's method, we obtained nail-cystine values slightly lower in some normal subjects than did Sullivan. The majority of all arthritics studied had normal content of cystine in their nails, using our normal values or Sullivan's as the criterion.

Examination of Table VII shows that several of the arthritics whose sulfur metabolism was studied had low finger-nail cystine values. Their basal sulfur excretion, however, was no different from that of subjects with normal nail-cystine or controls (see Table I). Furthermore, the sulfur excretion during and after the administration of colloid sulfur and sulfur-containing salts was no different in those subjects with low cystine content of finger-nails (Tables II through V). Repeated examinations in all subjects showed surprisingly constant cystine values (whether the initial content was low or high) and in no case was there a significant increase in the cystine content of the nails after the administration of large amounts of sulfur, even in those with initially low cystine content.

These data lead to the conclusion that: *No significant increase occurs in the content of cystine in finger-nails of persons treated with large amounts of colloidal sulfur or sulfur-containing salts. This is further evidence that sulfur administered in this way does not prevent or correct any deficiency of sulfur.*

DISCUSSION

One of the most interesting and consistent results of our investigation was that, during the pe-

TABLE VII

The cystine content of the finger-nails of subjects of metabolism study

Subject	Sulfur administered		Nails obtained	Cystine
		mgm.		*per cent*
Normals:				
W. A.	November 11, 1938 to December 2, 1938	1503	November 5, 1938	11.32
			December 6, 1938	11.40
			February 24, 1939	11.36
			April 5, 1939	11.34
D. Mc.	March 9, 1939 to March 30, 1939	1616	March 7, 1939	9.81
			April 13, 1939	9.78
			May 17, 1939	9.79
			July 31, 1939	9.82
H. M.	October 24, 1938 to October 26, 1938	260	October 4, 1938	9.81
			November 21, 1938	9.78
			December 3, 1938	9.73
E. W.	April 24, 1939 to May 12, 1939	830	April 21, 1939	11.30
			May 16, 1939	11.33
			May 23, 1939	11.37
	Initial nail cystine range 9.81 to 11.32 per cent			Average 10.56
Rheumatoid arthritics:				
C. B.	October 21, 1938 to December 1, 1938	2146	October 21, 1938	10.32
			December 12, 1938	10.28
			January 12, 1939	10.33
			March 1, 1939	10.31
O. J.	December 27, 1938 to January 28, 1939	2146	December 24, 1938	11.73
			February 4, 1939	11.68
			February 27, 1939	11.70
			April 1, 1939	11.69
			May 5, 1939	11.72
			June 26, 1939	11.73
			July 17, 1939	11.70
G. Mc.	February 16, 1939 to March 18, 1939	1866	February 2, 1939	9.31
			February 28, 1939	9.28
			May 8, 1939	9.62
			June 7, 1939	9.46
			July 26, 1939	9.37
S. K.	January 4, 1939 to February 5, 1939	2146	January 16, 1939	10.32
			February 15, 1939	10.29
			March 18, 1939	10.27
			October 9, 1939	10.28
	Initial nail cystine range 9.31 to 11.71 per cent			Average 10.47
Hypertrophic arthritics:				
G. M.	June 19, 1939 to July 9, 1939	1430	June 13, 1939	8.79
			July 16, 1939	8.73
D. M.	May 9, 1939 to May 24, 1939	2130	May 27, 1939	10.12
			June 29, 1939	10.14
			July 3, 1939	10.15
			October 5, 1939	10.11
	Initial nail cystine range 8.79 to 10.12 per cent			Average 9.45
Spondylitis rhizomelicas:				
S. G.	July 3, 1939 to July 21, 1939	1430	June 29, 1939	9.31
			July 26, 1939	9.34
			October 6, 1939	9.32
J. S.	April 6, 1939 to May 1, 1939	1090	April 2, 1939	10.61
			May 6, 1939	10.58
	Initial nail cystine range 9.31 to 10.61 per cent			Average 9.96

TABLE VIII

Cystine content of finger-nails from persons whose sulfur metabolism was not studied

Subject	Sex	Diagnosis	Nail cystine
			per cent
W. P.....	M	No disease	11.89
C. S.....	M	No disease	11.58
W. B.....	M	No disease	11.63
R. F.....	M	No disease	11.32
M. H.....	F	No disease	10.12
Cystine range..		10.12–11.89 per cent	Average 11.31
L. S.....	F	Rheumatoid arthritis	10.32
H. J.....	M	Rheumatoid arthritis	10.32
J. S.....	M	Rheumatoid arthritis	11.73
R. J.....	M	Rheumatoid arthritis	10.82
A. T.....	M	Rheumatoid arthritis	6.73
N. P.....	F	Rheumatoid arthritis	9.32
G. F.....	M	Rheumatoid arthritis	11.73
L. C.....	F	Rheumatoid arthritis	10.43
E. S.....	F	Rheumatoid arthritis	11.10
Cystine range..		6.73–11.73 per cent	Average 10.28
C. J.....	M	Mixed arthritis	11.01
M. W....	F	Mixed arthritis	11.20
			Average 11.10
N. T.....	M	Arthropathia psoriatica	8.71
F. B.....	F	Arthropathia psoriatica	12.12
			Average 10.41
M. W....	M	Spondylitis rhizomelica	10.63
R. H.....	M	Spondylitis rhizomelica	9.93
			Average 10.28

riod when colloidal sulfur was injected, the urinary excretion of sulfur increased by amounts greater than the amount of sulfur injected. We cannot explain this. This same finding was reported by Meyer-Bisch (19). He thought that it might have resulted from tissue destruction at the site of the injection of the oil suspension into the muscle because the nitrogen excretion also increased. In his patients fever occurred after the injections, possibly because of muscle destruction, or as a direct result of the medication. In either case, increase in protein catabolism could account for the excesive excretion of both nitrogen and sulfur. Fever did not occur after intramuscular injection in any of our subjects. We observed the excessive excretion of sulfur with the injection of colloidal sulfur intravenously as well as intramuscularly. Also, non-sulfur-containing fluid injected intramuscularly caused no increase in nitrogen or sulfur excretion. In only one patient, when sulfur was injected, did nitrogen excretion increase about parallel to the increase in sulfur elimination so that the N/S ratio definitely decreased. These observations would indicate that no appreciable muscle destruction occurred in our subjects.

Another consistent observation was that the increased excretion of sulfur, which occurred when the various sulfur preparations were given by injection or orally, was chiefly in the inorganic sulfate fraction. Lewis (20) points out that this is the normal route of elimination of added sulfur. Recently Greengard and Woolley (21) reported that colloidal sulfur fed to rabbits and to humans was eliminated almost entirely as inorganic sulfate.

Our results indicate that if it should be desirable to give sulfur as medication, there is certainly no biochemical or metabolic advantage in injecting it either intramuscularly or intravenously. Our results, however, show no metabolic need for sulfur administration. It is interesting to note in this regard that Senturia (6) in studying urinary sulfur excretion, found the same, and that Wheeldon (7), who is one of the most ardent advocates of colloidal sulfur therapy in arthritis, found no evidence of benefit from sulfur injection in his blood studies of sulfur and sulfur compounds.

The significance of low cystine content of finger-nails is not clear. Not all patients with arthritis have low nail cystine; many have entirely normal values. Moreover, low nail cystine is not peculiar to arthritis. It has been observed in patients chronically ill with other diseases (pellagra, tuberculosis, etc.). Malnutrition may account for the low values in all instances. Different authors have reported that after injecting colloidal sulfur into arthritics, the cystine content of the nails increased when it had been low, and frequently it became entirely normal. Some clinicians (li) have stated that best results from sulfur therapy occur in those patients with low cystine content of the nails. Comroe (22) recently stated that he noticed no definite correlation between improvement in the arthritis and increase in the cystine content of the nails, nor could he predict on the basis of nail cystine which patients would benefit most by colloidal sulfur therapy.

There is no evidence that colloidal sulfur or inorganic sulfate introduced into the body in any way can be used for synthesis of sulfur-containing amino acids (20). The analyses of nails from

our metabolism subjects show no significant change in the cystine content after large amounts of sulfur medication.

Forbes and Neale (14) report that thirty-four of thirty-nine patients with arthritis whom they studied excreted indole in the urine. When sulfur was administered, the arthritis improved and indoluria disappeared. Eight of our metabolism subjects studied daily by the same method used by Forbes and Neale did not have indoluria at any time, except for one patient who had a trace on two days only. It appears from our results that indoluria is certainly not common in arthritics.

Our investigation was purely biochemical and metabolic; no specific observation of clinical changes in our metabolism subjects was made. We wish to call attention to the fact that often when benefit was attributed to the parenteral use of colloidal sulfur, fever was produced by the injection. It is now well known that fever from whatever cause is often temporarily beneficial to arthritis. This benefit should not be attributed to a specific effect of sulfur.

SUMMARY

Our investigations show that there is no important difference in the amount of sulfur eliminated by patients with arthritis as compared to normal individuals. We found no indication of sulfur deficiency in any patient with arthritis. The amount of sulfur eliminated in each urinary fraction was practically identical in the arthritics and controls, except for a slightly higher conjugated fraction in the arthritics. This difference is most likely unimportant; if it is significant, it clearly indicates there is a sufficient supply of sulfur available for conjugation and that there is no impairment of this detoxification mechanism.

The injection of colloidal sulfur effected the same changes in patients with arthritis and in normal individuals: When injected intravenously the sulfur excretion in all but one normal subject and one patient increased by amounts considerably greater than the amount of sulfur injected; when injected intramuscularly, the sulfur excretion in *every* subject was increased by amounts *much* greater than the amount injected. Thus, the injection of colloidal sulfur actually tended to create a sulfur deficiency. Obviously, then, this method of treatment could not be expected to prevent or diminish a deficiency of sulfur in the body if such deficiency existed. Injected sulfur was eliminated chiefly as inorganic sulfate; except in one of the eight arthritics there was no increase in the conjugation of sulfur. Hence, rarely if ever, could injected colloidal sulfur be beneficial because of conjugation with toxic substances.

Sulfur given orally in colloidal form and as sodium thiosulfate effected the same changes in the arthritics as in the normal individuals; there was no increase in conjugation of sulfur.

Thymol given orally in capsules was readily conjugated with sulfuric acid by the arthritics and the controls similarly, thus providing further evidence that there is no impairment of this detoxifying mechanism and no need for sulfur medication on this account. Except for traces found in one patient on only two days, daily analysis showed no indoluria in eight subjects.

There is no biochemical evidence that *elemental* sulfur or inorganic sulfate injected or ingested can be utilized by the human in the synthesis of sulfur-containing amino acids. Our analysis of fingernails showed no significant changes in the cystine content after sulfur medication.

CONCLUSIONS

1. No evidence of sulfur deficiency or abnormality of sulfur metabolism was found to exist in patients with arthritis.

2. The data of this study reveal no *biochemical* or *metabolic* indication of need for, or benefit from, sulfur medication in the treatment of arthritis.

The authors are indebted to Miss Adelia Beeuwkes and her staff for the preparation of all meals during this investigation.

BIBLIOGRAPHY

1. a. Cawadias, A., Therapeutic action of colloidal sulfur. Bull. Acad. de méd., Paris, 1917, **78**, 329.
   b. Comrie, J. D., Treatment of trench rheumatism and allied conditions by colloidal sulphur. Lancet, 1917, **1**, 991.
   c. Rohde, E., Behandlung des chronischen Gelenkrheumatismus mit Schwefelinjektionen, Abstract. Klin. Wchnschr., 1922, **1**, 864.
   d. Schlesinger, H., Erweiterung der Indikationen fur die Schwefeltherapie bei innerin Erkrankungen. München. med. Wchnschr., 1931, **78**, 1300.

e. Woldenberg, S. C., Sulfur (colloidal) therapy in treatment of arthritis, with report of 100 cases. M. Rec., 1934, **139**, 161.
f. Woldenberg, S. C., Treatment of arthritis with colloidal sulfur: report of 250 cases. South. M. J., 1935, **28**, 875.
g. Argy, W. P., Arthritis: treatment with sulfur by intravenous and intramuscular injection. J. Bone and Joint Surg., 1934, **16**, 909.
h. Senturia, B. D., Results of treatment of chronic arthritis and rheumatoid conditions with colloidal sulfur. J. Bone and Joint Surg., 1934, **16**, 119.
i. Rawls, W. B., Gruskin, B. J., and Ressa, A. A., Value of colloidal sulfur in treatment of chronic arthritis. Am. J. M. Sc., 1935, **190**, 400.
j. Parmenter, D. C., Sulfur therapy in arthritis: preliminary report of some clinical results. Kentucky M. J., 1937, **35**, 14.
k. Wheeldon, T. F., The use of colloidal sulfur in the treatment of arthritis. J. Bone and Joint Surg., 1935, **17**, 693.
l. Fletcher, E., Value of sulfur and iodine in treatment of chronic arthritis. J. Roy. Inst. Pub. Health and Hyg., 1939, **2**, 104.

2. a. Reimann, H. A., and Pucher, G. W., Use of sulfur in treatment of arthritis deformans. Am. J. M. Sc., 1924, **168**, 77.
b. Cecil, R. L., Medical treatment of chronic arthritis. J. A. M. A., 1934, **103**, 1583.
c. Krestin, D., Treatment of chronic non-specific arthritis with intramuscular injections of sulfur. Brit. M. J., 1935, **2**, 1144.
d. Kinsella, R. A., Medical aspects of chronic arthritis. Radiology, 1935, **24**, 413.
e. Thompson, H. E., Wyatt, B. L., and Hicks, R. A., Chronic atrophic arthritis. Ann. Int. Med., 1938, **11**, 1792.
f. Dawson, M. H., Chronic Arthritis, in Nelson's Loose Leaf Medicine, Thomas Nelson and Sons, New York, 1935, **5**, 605.
g. Holbrook, W. P., and Hill, D. F., Treatment of atrophic arthritis. J. A. M. A., 1936, **107**, 34.

3. Debus, H. J., Chemical investigation of Wachenroder's solution, and explanation of the formation of its constituents. J. Chem. Soc., London, 1888, **53**, 278.

4. Goldthwait, J. E., Painter, C. F., and Osgood, R. B., The preliminary report of a series of metabolism observations made in atrophic arthritis, hypertrophic arthritis, osteitis deformans and the normal. Am. Med., 1904, **7**, 590.

5. American Medical Association, Council on Pharmacy and Chemistry, Report: Colloidal sulfur in the treatment of chronic arthritis. J. A. M. A., 1938, **111**, 1657.

6. Senturia, B. D., Urinary sulfur in chronic non-specific arthritis. J. Lab. and Clin. Med., 1935, **20**, 855.

7. Wheeldon, T. F., and Bosher, L. H., A biochemical investigation of arthritis; preliminary report on uric acid glutathione, and sulfur in blood; and sulfur in urine. Am. J. Surg., 1939, **43**, 598.

8. Sullivan, M. X., and Hess, W. C., Cystine content of finger nails in arthritis. J. Bone and Joint Surg., 1934, **16**, 185.

9. Loeper, M., Mahondeau, D., and Tonnet, J., Le metabolisme dans le rhumatisme generalise chronique progressif. Conference Scientifique Internationale du Rhumatisme Chronique Progressif Generalise (Aix-Les-Bain), 1934, **1**, 92.

10. Folin, O., Approximately complete analyses of thirty "normal" urines. Am. J. Physiol., 1905, **13**, 45.

11. a. Benedict, S. R., The estimation of total sulphur in urine. J. Biol. Chem., 1909, **6**, 363.
b. Denis, W., The determination of total sulphur in urine. J. Biol. Chem., 1910, **8**, 401.

12. Rossouw, S. D., and Wilken-Jorden, T., Studies on the origin of the sulphur in wool. I. A study of the Sullivan technique for cystine. Ond. J. Vet. Sci. Animal Ind., 1934, **2**, 361.

13. Folin, O., On the determination of creatinine and creatine in urine. J. Biol. Chem., 1914, **17**, 469.

14. Forbes, J. C., and Neale, R. C., Studies on indoluria. J. Lab. and Clin. Med., 1935, **20**, 1017.

15. Freyberg, R. H., and Grant, R. L., Loss of minerals through the skin of normal humans when sweating is avoided. J. Clin. Invest., 1937, **16**, 729.

16. Forbes, J. C., and Neale, R. C., The production of chronic arthritis by indole and other products of tryptophane putrifaction. J. Lab. and Clin. Med., 1937, **22**, 921.

17. Foster, C. S., and Kahn, M., A study of the tests of liver function. J. Lab. and Clin. Med., 1916, **2**, 25.

18. Klauder, J. V., and Brown, H., Sulfur content of hair and of nails in abnormal states; nails. Arch. Dermat. and Syph., 1935, **31**, 26.

19. a. Meyer-Bisch, R., and Basch, E., Über das Schicksal parenteral verabreichten Schwefels und seinen Einfluss auf den Stoffwechsel. Biochem. Ztschr., 1921, **118**, 39.
b. Heubner, W., and Meyer-Bisch, R., Über Sulfat- und Esterschwefelsäure in normalen und pathologischen Körperflüssigkeiten. Biochem. Ztschr., 1921, **122**, 120.

20. Lewis, H. B., Sulfur metabolism. Physiol. Rev., 1924, **4**, 394.

21. a. Greengard, H., and Woolley, J. R., Absorption and oxidation of orally administered colloidal sulphur. Am. J. Physiol., 1939, **126**, 511.
b. Greengard, H., and Woolley, J. R., Studies on colloidal sulfur-polysulfide mixture: absorption and oxidation after oral administration. J. Biol. Chem., 1940, **132**, 83.

22. Comroe, B. I., Sulfur therapy in arthritis; with review of literature. Medicine, 1939, **18**, 203.