previous day. Both of these specimens on the tellurite-iron-rosolic acid medium showed hundreds of colonies of Flexner's bacillus and absolutely no *B. coli*, whereas the same emulsion on MacConkey's plates yielded a profuse growth of *B. coli* and no recognizable colonies of *B. dysenteriae*.

Summary

Lactose nutrient agar containing, per 100 c.cm., 0.5 c.cm. of a 1% alcoholic solution of rosolic acid, 0.5 c.cm. of a 1% solution of potassium tellurite, and 1 c.cm. of a 4% solution of iron alum provides a medium on which Flexner's *B. dysenteriae* grows profusely, whereas there is great inhibition of most strains of *B. coli* and *B. lactis aerogenes*. The growth of *Salmonella* food-poisoning organisms, typhoid and paratyphoid bacilli, Sonne's bacillus, and cholera vibrios is suppressed.

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A NEW SULPHONAMIDE (SULPHONAMIDE E.O.S.)

ITS PHARMACOLOGY, CHEMOTHERAPY, AND CURATIVE VALUE IN MENINGOCOCCAL MENINGITIS

BY

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Although some thousands of sulphonamide derivatives have now been prepared and investigated, very few have displayed any outstanding therapeutic qualities which would justify their general adoption in preference to the simple parent substance sulphanilamide or its popular variant sulphapyridine. The clinician desires something more than an active chemotherapeutic agent. He asks for the means of easy administration to patients of all ages and in all conditions and for freedom from side-actions which are either distressing or dangerous. The new compound employed in this investigation* is freely soluble in water, and 50% w/v solutions are easily obtained. It dissolves to form an almost tasteless preparation with a faintly acid reaction which can be reduced to one of absolute neutrality without precipitation or loss of stability. The treatment of infants and of fastidious, comatose, vomiting, or diarrhoeal patients is therefore easily carried out with solutions given by mouth, nasal tube, rectal tube, or intravenous drip. The familiar 0.5 gramme tablet can be reserved for routine use. In these respects it contrasts favourably with sulphanilamide, which is relatively insoluble in water, with sulphapyridine, which is much more insoluble still, and with sulphapyridine sodium, which, although freely soluble, forms a strongly alkaline solution approximately equivalent in reaction to decinormal caustic soda. Sulphonamide E.O.S. possesses the further attractive feature of low toxicity, and its administration does not lead to the formation of such highly insoluble bodies as the acetylated compounds, which tend to crystallize in the urinary passages when sulphapyridine or sulphathiazole is used (Leedham-Green, 1941).

* The material used in this investigation was supplied by the Research Department of Imperial Chemical Industries Ltd.

Chemistry and Fate in the Body

The substance was first described by Green and Coplans (1940), who identify it as sulphonamide ethyl-alpha-sulphonate,

The side chain was chosen with two deliberate intentions—namely, that of reducing the basicity of the original sulphanilamide and so reducing its toxicity, and that of furnishing a link (as indicated by the arrows) which could easily be broken in the body by hydrolysis, with the liberation of the parent nucleus. In aqueous solution it is stable at room temperature, but hydrolysis occurs on boiling, with the formation of sulphanilamide, acetaldehyde, and sodium bisulphite. Decomposition proceeds more quickly in the presence of acids or alkalis, and is accelerated very greatly indeed by oxidizing reagents such as iodine or hydrogen peroxide. Hydrolysis at body temperature and at pH concentrations which are known to occur in the alimentary tract is relatively slow. The pertinent data given in Table I were furnished by the late Prof. A. G. Green.

TABLE I.—Hydrolysis of Sulphonamide E.O.S.

Reaction of Solution (2½% E.O.S.)	% E.O.S. hydrolysed in 3 Hours at 37° C.
N/10 HCl (hyperchlorhydria)	4.0
N/20 HCl (normal test-meal peak) ..	2.8
Neutral	0.7
0.2% NaHCO ₃	1.8

Throughout this range more than 95% of the drug remains unaffected at the end of three hours, and during this period a large proportion of the drug is absorbed. After absorption hydrolysis proceeds much more rapidly.

A 50% solution w/v was injected intravenously (dose 5 c.cm. = 2.5 grammes E.O.S.). Of this only 16% could be recovered unchanged from the urine, and even during the first thirty minutes about one-half was excreted as sulphanilamide or as acetyl-sulphanilamide, the side chain having been removed from the original drug (Table II).

TABLE II.—Intravenous Administration of Sulphonamide E.O.S. (Man). (Dose, 2.5 grammes in 5 c.cm.)

Interval after Administration (Hours)	Rate of Urinary Excretion	
	Unsplit Sulphonamide E.O.S. (Mg. per ½ hour)	Total Sulphonamide, expressed as Sulphanilamide (Mg. per ½ hour)
0-½	220	471
½-1	140	317
1-1½	40	—
1½-2	10	—
2-5	0	69
5-6	0	115

TABLE III.—Administration of Sulphonamide E.O.S. by Mouth (Man). Dose, 4 grammes dissolved in water taken between meals at 11 a.m.)

Interval after Administration	Vol. of Urine (c.cm.)	Rate of Urinary Excretion	
		Unsplit Sulphonamide E.O.S.	Free Sulphonamide, expressed as Sulphanilamide (mg. per ½ hour)
0-20 minutes	15	Small amount	Trace
20-45 "	15	" "	3.0
45-60 "	15	" "	13.4
1-1½ hours	25	" "	15.5
1½-2 "	25	None	17.0
2-3 "	35	" "	12.0
3-5 "	55	" "	11.8
5-7 "	50	" "	8.6
7-9½ "	60	" "	9.2

The excretion of sulphonamide E.O.S. ceased after two hours, but excretion of considerable amounts of sulphanilamide continued for many hours. The rate of excretion was substantially modified by variations in urinary flow. Excretion was decidedly more rapid during water diuresis. This rapid hydrolysis in the

body can be ascribed provisionally to the presence of oxidizing agents such as tissue oxidases and oxyhaemoglobin.

When a single dose is given by mouth, excretion of unsplit E.O.S. begins within twenty minutes and continues for an hour and a half, after which time E.O.S. can no longer be found in the urine, but sulphanilamide and its acetylated derivative are excreted in substantial amounts throughout the day. The peak rate of total sulphonamide excretion occurs between one and a half and two hours after administration of the drug (Table III). Of the original 4 grammes only about 30 mg. could be found in the urine in its original form. Hydrolysis is therefore almost complete.

With the slower rate of absorption following administration by rectum it was impossible to find any unsplit E.O.S. in the urine, and the peak rate of sulphanilamide excretion was much delayed (Table IV). Nevertheless the drug is therapeutically effective when given in this way (Case 19, Table VIII).

TABLE IV.—*Rectal Administration of Sulphonamide E.O.S. (Man). (Dose, 4 grammes in 8 c.cm.)*

Interval after Administration (Hours)	Vol. of Urine (c.cm.)	Rate of Urinary Excretion	
		Unsplit Sulphonamide E.O.S.	Total Sulphonamide expressed as Sulphanilamide (Mg. per $\frac{1}{2}$ hour)
0– $\frac{1}{2}$	55	Nil	17
$\frac{1}{2}$ –1	30	"	12
1–1 $\frac{1}{2}$	23	"	21
1 $\frac{1}{2}$ –2	47	"	19
2–3	160	"	26
3–4 $\frac{1}{2}$	1,150	"	41
4 $\frac{1}{2}$ –5	230	"	23

During routine administration of the drug by mouth the unsplit sulphonamide E.O.S. appears in the cerebrospinal fluid (Table V) together with a preponderance of sulphanilamide.

TABLE V.—*Cerebrospinal Fluid (after Administration of Sulphonamide E.O.S. by Mouth)*

Case	Age	Illness	Dose	Lumbar Puncture Performed	Interval between Lumbar Puncture and Most Recent Dose of E.O.S.	Concentration of Drug in C.S.F.	
						Unsplit E.O.S.	Total Sulphonamide, expressed as Sulphanilamide
A	6	Cerebro-spinal meningitis	Repeated 2 gm. four-hourly	2nd day	Hours 3 $\frac{1}{2}$	Trace	Mg. % 8
					4	"	10
					1 $\frac{1}{2}$	"	16
B	32	Epilepsy	Single Dose 4 gm. fasting		$\frac{1}{2}$	Nil	1.6
C	31	"	"		1	"	Trace
D	26	"	"		1 $\frac{1}{2}$	"	2.6
E	15	"	"		2	"	1.2
F	73	"	"		2 $\frac{1}{2}$	"	Trace

The analytical methods used were:

Sulphanilamide + E.O.S. = free sulphonamide
 Sulphanilamide + E.O.S. + acetylated sulphanilamide = total sulphonamide

} Marshall's method (Marshall et al., 1937)

Unsplit sulphonamide E.O.S.:

The following method was devised by A. G. Green: To 25 c.cm. of urine add 5 c.cm. of 10% barium chloride solution and filter. To filtrate add 2 c.cm. of concentrated hydrochloric acid and filter again if necessary. To the clear solution add 1 c.cm. of N/1 sodium nitrite (7.2% solution). If free sulphonamide E.O.S. is present a further turbidity or precipitate will form and can be compared with a standard control.

Conclusions.—Sulphonamide E.O.S. is probably adsorbed unchanged when given by mouth or rectum. In the body it is rapidly hydrolysed, but during therapeutic schemes of routine four-hourly doses small amounts of unsplit drug persist in the cerebrospinal fluid throughout the inter-dose period. When administered orally it is excreted in the urine to a small extent unchanged, but mainly in the form of sulphanilamide with a small proportion of the acetylated

derivative of sulphanilamide. On intravenous injection hydrolysis to the extent of 25% occurs within an hour and is complete after two hours. Opportunities for an individual therapeutic action are offered by (1) the production of sulphanilamide *in situ* in the blood and tissues, and possibly in the bacterium itself; (2) the independent action of the unsplit drug; (3) the rapid rate of absorption from the upper alimentary tract and rectum.

Experimental Therapeutics

The toxicity of sulphonamide E.O.S. has been assessed in terms of lethal dose administered to animals by mouth and by clinical survey of phenomena appearing during therapeutic administration in man. Values given by various observers for the toxicity of sulphanilamide measured in terms of L.D.50 range between 60 and 80 mg. for the 20-gramme mouse. Such figures are influenced by the strain of mouse used. In the present research female albinos were employed and parallel doses of sulphanilamide suspension and sulphonamide E.O.S. solution were administered by oesophageal tube. Deaths were recorded seventy-two hours later, when surviving animals appeared to be normal. For sulphanilamide L.D.50 was found to be 70 mg. per 20-gramme mouse. For sulphonamide E.O.S. it was 200 mg., giving a toxicity ratio on a gravimetric basis of about 1:3 in favour of sulphonamide E.O.S. The relative molecular weights of the two substances must be taken into consideration:

$$\frac{\text{Mol. wt. E.O.S.}}{\text{Mol. wt. sulphanilamide}} = \frac{302}{172}; \text{ i.e., approximately } \frac{7}{4}$$

7 grammes of E.O.S. are therefore equivalent in nuclear content to 4 grammes of sulphanilamide. Making the appropriate correction, the toxicity ratio becomes 1:1.6, showing that the amount of sulphonamide nucleus which can be given in the form of sulphonamide E.O.S. is substantially greater than that which can be given with equal safety in the form of sulphanilamide. Further toxicity tests and an experimental investigation of the curative action of the drug on infected laboratory animals were carried out by Dr. M. Coplans as follows:

(i) Acute Infections

Streptococcus pyogenes (from the National Collection of Type Cultures Group A—Griffith No. 5493)

Albino mice, and doses calculated per 20 grammes weight: 5 million streptococci injected intraperitoneally; 1 c.cm. 5% aqueous solution (neutral) E.O.S.=25 mg., given subcutaneously 1/2 hour after inoculation and repeated daily.

21 mice inoculated and treated:

3, duration of treatment 2 days. All died on ninth day.

18, duration of treatment 10 to 20 days. 11 survived, and cultures taken between 46th and 58th day were all negative.

Controls:

18 mice inoculated and not treated. All dead within 17 $\frac{1}{2}$ hours and organism recovered from heart blood and peritoneal fluid.

18 mice treated for 10 to 20 days but not inoculated. 15 survived (3 died on 7th, 18th, and 27th days).

Pneumococcus Type III (from the National Collection of Type Cultures Type III—Griffith No. 2438)

Albino mice, and doses calculated per 20 grammes weight: broth culture (100 $\times$ M.L.D.) intraperitoneally; 0.5 c.cm. aqueous solution of E.O.S.=166 mg., given by mouth 1/2 hour after inoculation and repeated daily.

6 mice inoculated and treated for 5 days. All alive and well 9 weeks later.

Controls:

6 mice inoculated and not treated. All dead within 60 hours and organism recovered from heart blood and peritoneal fluid.

6 mice treated for 5 days but not inoculated. All alive and well 9 weeks later.

(ii) *Urinary Carriers*

Immature Dutch rabbits were used and the carrier condition induced by Coplans's (1940) method.

B. typhosus:

7 carriers. Treatment started on 19th day. Sulphonamide E.O.S. 2 grammes per kilogramme by mouth alternate days for 5 doses and then daily for a further 33 doses (to surviving animals). In every case the bladder and urine became free from infection.

6 untreated carriers. 1 recovered spontaneously. In 5 infection persisted in the bladder or urine.

B. paratyphosus A:

5 carriers. Treatment started on 40th day. Sulphonamide E.O.S. 2 grammes per kilogramme by mouth daily for 45 doses. In every case the bladder and urine became free from infection.

6 untreated carriers. Infection of bladder and urine persisted in all animals.

Conclusions.—Sulphonamide E.O.S. controls acute infections by *Str. pyogenes* and pneumococcus Type III in mice and chronic infections of the urinary carrier type due to *B. typhosus* and *B. paratyphosus A* respectively in rabbits.

Clinical Observations

The clinical data relating to the toxicity of sulphonamide E.O.S. are derived from 130 cases treated for a variety of acute infections. The doses used in different age groups are given in Table VI.

TABLE VI.—*Sulphonamide E.O.S. Dosage (Clinical Toxicity Test) (130 Cases)*

Group	No.	Average Age in Years	Dosage in Grammes					
			Initial		1st 3 days		Total	
			Range	Average	Range	Average	Range	Average
Infants* (up to 12 months)	27	6/12	1-1	0.8	1½-18	10.3	2½-67	21.7
Children (1 to 10 years) ..	31	3½	1-3	1.5	2-45½	19.0	5-82½	36.7
Adolescents (10 to 20 years) ..	17	16	2-4	2.5	12-49	29.3	12-72	44.6
Adults (above 20 years) ..	55	—	1-4½	2.6	9-56½	32.0	15-100	51.9

* The youngest infant, aged 3 weeks, was given an initial dose of 1 gramme, 11½ grammes in three days, and 17½ grammes *in toto*. When first seen she was extremely ill with bronchopneumonia, and artificial respiration was required. An oxygen tent and α -lobeline injections were used. The condition came steadily under control in the course of a few days. No toxic signs attributable to the drug were noted in spite of the exceptionally heavy dosage adopted.

The general malaise of sulphonamide therapy was very little in evidence, and that miserable sequence of pallor, nausea, and vomiting characteristic of sulphapyridine never occurred. In many cases (not included in Table VI) treatment was instituted with sulphapyridine and subsequently completed with sulphonamide E.O.S. The general appearance and subjective feeling of severe illness of these patients disappeared rapidly when the substitution was made. The visible change for the better on E.O.S. therapy was very remarkable indeed.

Cyanosis was commonly seen, but never caused any distress or necessitated the interruption of the prescribed course:

Absent	40%
Trivial	28%
Substantial	29%
Severe	3%
Requiring withdrawal of drug	0%

Vomiting was rarely encountered. In pregnancy and meningitis vomiting often occurs without provocation. If such cases are excluded the trouble was confined to a small minority of infants and young children in whom the causal relation between the drug and the vomiting was by no means clear.

Leucopenia.—Routine blood counts were made without bringing to light any instance of agranulocytosis or simple

leucopenia. The leucocytes were never fewer than 6,000 per c.mm. of peripheral blood, with the exception of one case in which the white count fell to 5,400 cells per c.mm.

Anaemia.—Minor degrees of anaemia were found, but not with greater frequency or of greater severity than is usual after severe infective illnesses. No severe anaemia of aplastic or of haemolytic type was ever observed.

Rashes.—One patient developed a simple erythematous rash—a fine pink widely distributed eruption occurring in a young child after a week of treatment with E.O.S.

A curious case of acidosis (Case 25, Table VIII) occurred in a young woman of 28 who was semicomatose from meningococcal meningitis. There were no ketone bodies in the urine, but the alkaline reserve of the blood was low. In terms of CO₂ binding power it varied between 34.8 vols. CO₂% and 36 vols. CO₂% respectively over two successive days (normal value, 53 to 77). The inorganic blood phosphorus remained within normal limits. The pH of the urine fell to 5.0 (analyses by Dr. S. J. Hopkins). The outstanding clinical feature was pronounced hyperventilation beginning at the end of the second day of treatment, by which time 40 grammes of sulphonamide E.O.S. had been taken. It persisted for three days, and was unaffected by oxygen administration (B.L.B. mask). There was no cyanosis. Hyperventilation subsided when full doses of alkalis were given. Administration of E.O.S. continued uninterrupted, and its curative effect as judged by the mental condition, defervescence, and observations in the cerebrospinal fluid was not prejudiced. The temperature was normal within 7 days.

Although the case must be put on record it is by no means certain that the abnormality was caused by the drug, and the complication might possibly have been prevented by a freer use of routine alkalis, always advisable for febrile patients taking sulphonamides.

The general inference to be drawn from these observations is very satisfactory indeed. In spite of the heavy doses given and the cyanosis produced, there were no serious complications, and the assortment of symptoms which distress without endangering the patient when sulphapyridine is used were conspicuously absent.

Chemotherapeutic Effect (Human)

The drug has now been used in a large number of different clinical conditions with apparently excellent effect—e.g., acute *B. coli* infections of the urinary tract, puerperal infection, septic cuts, acute infections of ear, nose, and throat, pneumonia (including pneumococcal and Friedländer infections, the mixed bronchopneumonias of infancy), and septic pleuritic effusions. The expected recovery rate in these disorders is reasonably high even when non-specific therapy alone is carried out. The prognosis varies greatly from case to case. For these reasons it is extremely difficult to secure a datum line from which to judge the success or failure of any new curative method. More trustworthy conclusions can be formed by selecting more intractable

TABLE VII.—*Dosage of Sulphonamide E.O.S. (Acute Cerebrospinal Meningitis—Meningococcal)*

	Adults (Grammes)	Children (Grammes)			
		10 years	5 years	2 years	Infants
1st two doses	4	3	2½	1½	1
Remainder of first 2½ days	3	2½	2	1½	1
Next 1½ days	2-2½	1-1½	1-1½	1-1	1-1
5th day	1½-2	1-1	1-1	1-1	1-1
6th day	1-1½	1-1	1-1	1-1	1-1
7th day	1-1	0-1	0-1	0-1	0-1
8th day	0-1	0-1	0-1	0-1	0

diseases, such as chronic *B. coli* infections of the kidney and bladder or purulent meningitis, as the medium for therapeutic assay.

Attention has therefore been centred on acute epidemic cerebrospinal meningitis. The cases all occurred in the

Guy's Hospital Sector of the E.M.S. during 1940 and the early part of 1941. These patients were suffering from the disease in its acute form and many were very seriously ill indeed. Their ages ranged from 7 months to 63 years. About one-quarter were either less than 1 year or more than 45, and so fell into the age periods of gravest prognosis. The general dosage scheme is given in Table VII. Small variations were introduced to meet the needs of individual cases. The amount of sulphonamide used was not so great as these figures suggest. It must be remembered that 1 gramme of sulphonamide E.O.S. is equivalent chemically to no more than 0.57 gramme of sulphanilamide.

The outstanding clinical features of the cases are indicated in Table VIII. In every instance Gram-negative diplococci were seen in the cerebrospinal fluid. In most cases the organism grew in culture and many of them were typed. The commonest type was Group I, but some were not agglutinated either by Group I or Group II serum.

Case 19 is of particular interest. It demonstrates the efficacy of sulphonamide E.O.S. when administered by rectum. It also indicates that pregnancy does not interfere with the action of the drug and is not affected adversely by it. Normal labour occurred later at full term and the child was correctly formed and well nourished. The mother's health during the later months of pregnancy was excellent and the puerperium passed uneventfully. A considerable number of patients were comatose or semi-comatose on admission. In spite of the high proportion of infants and elderly persons and the coincident pulmonary diseases of Cases 2, 24, 26, and 34, the mortality rate of 9% is reasonably low. Analysis of the factors leading to death in the three fatal cases reveals special features in each case.

Case 1.—This was a neglected case, and chemotherapeutic treatment was not instituted until the third week of illness, when hydrocephalus was already established.

TABLE VIII

Case No.	Age Years	Treatment Day of Illness	C.S.F. (1st Lumbar Puncture)			Comment	Result
			Pressure mm.	Cells (Polymorphs per c.mm.)	Protein Per cent.		
1	7/12	20th				A neglected case in coma with hydrocephalus already established	Died in 48 hours
2	10/12	2nd	300	520	0.24	Intensely ill and unconscious. Had only recovered from pneumonia a month previously	Complete recovery 2½ weeks
3	11/12	14th	+	3,140	0.24	Slow onset. Convulsions just before admission	Complete recovery. Temp. normal in 4 days
4	1 3/12	3rd	+	9,000	0.30	Very severe case. Pneumonia present on admission	Complete recovery. Rapidly controlled, but tardy clearance
5	1 9/12	3rd	+	3,460	0.90	Full average severity. Semi-comatose	Complete recovery
6	1 10/12	3rd	300	1,840	0.10	Average severity	Inadequate initial dosage, recovery protracted but eventually complete
7	1 11/12	2nd	280	4,250	0.45		Complete recovery
8	3	2nd	250	2,140	0.16	" "	" "
9	5	4th	320	7,360	0.10	" "	" "
10	7	5th	290	2,000	0.65	" "	" "
11	7	5th	250	1,620	0.20	" "	" "
12	9	4th	280	3,290	0.38	Semi-comatose. Old mitral regurgitation.	" "
13	13	3rd	230	1,106	0.35	Profuse herpes	" "
14	14	2nd	260	4,380	0.29	Average severity	" "
15	16	5th	300	12,700	0.30	Abrupt onset. Delirium. Screaming. Semi-coma	" "
16	17	4th	290	7,200	0.25	Average severity	Complete recovery. Temp. normal in 3 days
17	19	4th	240	800	0.20	Full average severity	Complete recovery
18	20	1st	335	2,720	0.36	Semicoma. Petechial rash	Complete recovery. Temp. normal in 5 days
19	22	2nd	250	2,600	0.35	Pregnant, and comatose 5 days. Early medication entirely per rectum. 87 grammes administered in this way over period of 5½ days before patient able to take by mouth. Subsequent progress exceptionally rapid	Complete recovery. Five mths. later gave birth to healthy full-term child
20	24	3rd	200	2,280	0.22	Average severity	Complete recovery. Temp. normal in 4½ days
21	24	3rd	210	3,490	0.31	" "	Complete recovery. Temp. normal in 3 days
22	26	4th	300	10,000	0.50	Insidious onset. Delirious on admission but not comatose	Complete recovery
23	27	3rd	280	8,620	0.37	Full average severity	" "
24	28	2nd	320	19,000	0.56	Very severe case. Coma complete within 24 hours. Fat and difficult to nurse. Vomiting conspicuous. Fluids, food, and drugs by nasal tube	Died on 4th day of treatment. P.M.: meningitis clearing. Cause of death, purulent bronchitis
25	28	3rd	315	28,000	0.52	Very severe, with persistent coma. Hyperventilation and acidosis for several days. Severe facial herpes	Complete recovery. Control established steadily. Temp. normal in 6 days
26	34	6th	324	9,800	0.15	Average severity. Old fibroid phthisis with cavities (X-ray finding)	Complete recovery. Temp. normal in 2½ days
27	35	3rd	280	10,100	0.28	Severe illness. Semicomatoze for 2 days, but able to take fluids by mouth	Complete recovery
28	35	2nd	240	7,420	0.10	Average severity	Complete recovery. Terminated by crisis in 36 hours
29	40	4th	350	13,000	0.48	Very severe. Semicoma for several days. Blood transfusion on 5th day of treatment. Temperature normal 36 hours later	Complete recovery
30	46	3rd	320	11,900	0.30	Severe illness; delirium but no coma	" "
31	48	3rd	140+ (Pus too thick to register correctly)	16,000 rising to 84,000	1.50	Intensely ill, comatose, dyspnoeic, cyanosed, and finally hyperpyrexial. Drug administered by rectum	Died after 2 days' treatment. P.M.: suppurative meningitis and acute suppurative pericarditis. Section of heart examined for secondary invaders, but none seen
32	53	4th	210	5,600	0.27	Average severity. Acute monarthrits of rt. wrist, a complication on 2nd day of treatment, continued for a little time after subsidence of fever and cerebral symptoms	Complete recovery
33	56	5th	250	6,200	0.18	Average severity	" "
34	63	2nd	300	9,000	0.90	Severe attack. Semicoma, but able to take fluids by mouth. Acute bronchitis on admission. Bronchopneumonia as a complication after 2 days' treatment, but temp. normal a week later	" "

Case 24.—This patient appears to have died of a complication of the unconscious state, possibly due to inhalation of infected material, rather than from the meningitis itself.

Case 31 acquired a particularly lethal and rare complication—suppurative pericarditis.

There were no deaths among those who were seen during the first week of illness and who remained free from serious secondary infections of the viscera. For these reasons more than for the simple statistical fact of a low mortality rate on a limited series of cases, I feel justified in believing that sulphonamide E.O.S. has a pronounced curative action in this disease. Very diverse figures have been published by other observers using different remedial agents. This diversity is readily understood by anyone who has worked among these patients. The mortality rate is affected greatly by so many factors other than that of the chemotherapeutic potency of the drug employed. It varies with the epidemic, locality, age distribution, the speed with which the diagnosis is made, the amount of medical attention which can be focused on each patient, and, above all, with the perfection of the nursing care available. Superb nursing skill is required by delirious, comatose, or semiconscious patients. I know that I have been particularly fortunate in my house-physicians and nurses.

The figures given below furnish a suitable basis for comparison.

Epidemic 1914-17 (pre-sulphonamide):
5,306 cases; mortality 65%

These national figures were analysed by H. D. Rolleston (1919), who found in them evidence of a dwindling mortality as serum treatment came into more general use.

Present epidemic, first quarter, 1940 (sulphonamides widely used):
4,388 civilian cases; mortality 23.7%

Individual groups of smaller numbers but more unified medical control have yielded better results:

120 cases observed personally by Banks (1940) (sulphapyridine or other sulphonamides used in all cases):
Mortality 10%

154 cases at Wrexham (*Lancet*, 1940) (various doctors) (sulphonamides widely used):
Mortality 12.3%

The mortality in the present series treated with sulphonamide E.O.S.—34 cases (9%)—is therefore closely in accord with the current figures.

Summary

A new sulphonamide of low toxicity and full chemotherapeutic activity in acute cerebrospinal meningitis is described. Less critical clinical data lead one to expect that it will be equally effective in combating infections due to *B. coli* and haemolytic streptococci. More extended clinical observations will probably reveal other fields of utility. In animals it controls acute infections due to *Str. pyogenes* and pneumococcus Type III, and chronic urinary infection by *B. typhosus* and *B. paratyphosus* A. Its low toxicity has been investigated by experiment on mice. The rarity with which in therapeutic dosage it provokes unpleasant side-effects in man has been demonstrated by a clinical survey of 130 cases. It seldom causes vomiting, and malaise attributable to the drug is inconspicuous. It is hydrolysed in the body with the liberation of sulphamylamide *in situ*, and is excreted in forms which are far more soluble than those following the administration of sulphapyridine or sulphathiazole. It possesses the convenient property of ready solubility in water. Neutral and almost tasteless pharmaceutical solutions can easily be prepared for administration by mouth, rectum, or vein.

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THE SIZE AND VISIBILITY OF THE FILTERABLE OR VIRUS BODIES

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The filterable, so-called ultra-microscopic or elementary, bodies, generally spoken of as the viruses, are not easy to examine on account of their very small size and the difficulty of staining them; but fortunately they are nearly always present in enormous numbers, often in almost pure cultures. The measurement of such small bodies is very difficult by eye-piece or screw micrometer, and usually one has to resort to photography.

The Photographic Method of Measurement

Muir and Ritchie (1937) state:

"In the microscopical study of virus particles Barnard has utilized photography by ultra-violet light, which is of shorter wave-length than that of the visible rays, and thus has obtained greater microscopical resolution. By this method he has been able to demonstrate photographically virus particles of 0.075μ in diameter."

Let us compare this with the results obtainable by the use of the ordinary microscope and objectives in common use when these are employed correctly. The late Mr. Edward Milles Nelson (1909), who devoted the whole of his long life to the microscope and was undoubtedly the greatest authority on the subject, says: "Briefly, the method is to bring the object just to the point of invisibility by reducing the N.A. (numerical aperture), and then the correction for antipoint will be the measure of the object." I might add that one can determine the minimum visible of any objective by the following calculation: for white light of 45,300 waves per inch divide 1.041 by the N.A.; for green light of 50,000 waves per inch divide 0.942 by the N.A. of the objective. A full table of the values of the N.A. is given in Mr. Nelson's paper.

I have used this method of measuring by extinction for many years, employing for this purpose a Leitz $1/10^a$ fluorite objective fitted with an iris diaphragm graduated in N.A.s, immediately behind the back lens, and find it remarkably accurate and easy after one has had some practice.

From Nelson's table it will be seen that an objective of N.A. 1.4 will reveal a particle of 0.0744μ with white light and 0.0673μ with a green screen. Nelson later, in 1935, informed me that even greater visibility could be obtained by using a motor head-light bulb (3 amps 12 volts with a transformer, as suggested by Ivey-Cook), with the filament in focus, and a deep blue screen. Then the size of an object seen would be 0.841 divided by the N.A.; so that an objective of N.A. 1.3 would show a particle 0.065μ , and one of N.A. 1.4 a particle 0.06μ , in diameter. From these figures, given in much greater detail in a previous paper (Coles, 1929), ultra-violet microscopy does not seem very urgently needed.

Mr. Eliot Merlin (1929), the distinguished microscopist, in concluding his masterly article, says:

"In the writer's opinion it is of paramount importance that the true optical capacity of the ordinary worker's microscope, furnished with ordinary glass and fluorite achromatic or apochromatic object-glasses, should be clearly understood and not underestimated, this being the tool exclusively used by thousands of laboratory and technical workers all the world over. It cannot be too emphatically maintained that it is an experimentally absolute fact that an object-glass of 0.4 N.A. (i.e., a common