# SULPHETRONE: THERAPEUTICS AND TOXICOLOGY[1] [2]

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The discovery in these laboratories of the chemotherapeutic activity of diaminodiphenylsulphone (Buttle *et al.* 1937) stimulated a search for derivatives having the efficiency of the parent drug but not its undesirable toxicity. One derivative, tetrasodium 4:4-bis (γ–phenylpropylamino)–diphenylsulphone–α : γ : α′ : γ′–tetrasulphonate, given the registered name "sulphetrone," was first prepared in 1936 and attracted attention on re-examination in 1941 by reason of its freedom from toxicity and its potent antituberculotic activity.

Brownlee *et al.* (1948) have described its structure, chemical and physical properties, pharmacology, experimental therapy, and possible clinical uses; and Brownlee and Kennedy (1948a) have described its suppressive effect on progressive experimental tuberculosis in guineapigs. The drug was found to be bacteriostatic and, in common with other sulphones, incapable of eliminating the infective organism from animal tissue. In a second study Brownlee and Kennedy (1948b) have shown that sulphetrone is more efficient than "promin" in protecting the laboratory animal against experimental tuberculosis, and that it is synergic with streptomycin. A clinical evaluation, unfortunately restricted to a limited series, was made by Madigan *et al.* (1947). The chemical preparation of sulphetrone has been described by Gray and Henry (1936) and by Buttle *et al.* (1938).

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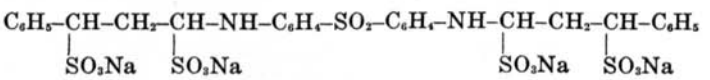
[1] Reprinted, with permission, from the *Lancet* **2** (1948) 131-134 (July 24). Other pertinent references are: MADIGAN, D. C., SWIFT, P. N. and BROWNLEE, G. Treatment of tuberculosis with streptomycin and sulphetrone. *Ibid.* **2** (1947) 897; ANDERSON, T. and STRACHAN, S. J. Chemotherapy of pulmonary tuberculosis with sulphetrone. *Ibid.* **2** (1948) 135; CLAY, M. G. and CLAY, A. C. Chemotherapy of tuberculosis with sulphetrone. *Ibid.* **2** (1948) 180; BROWNLEE, G., GREEN, A. F. and WOODBINE, M. Sulphetrone: A chemotherapeutic agent for tuberculosis; pharmacology and chemotherapy. British J. Pharm. & Chemoth. **3** (1948) 15-28; BROWNLEE, G. and KENNEDY, C. R. The treatment of experimental tuberculosis with sulphetrone. *Ibid.* **3** (1948) 29-36; BROWNLEE, G. and KENNEDY, C. R. The chemotherapeutic action of streptomycin, sulphetrone and promin in experimental tuberculosis. *Ibid.* **3** (1948) 37-43.

[2] The development of sulphetrone is part of a programme of work on antituberculous compounds carried out by the Therapeutic Research Corporation of Great Britain.

Sulphetrone is a cream-coloured amorphous material containing 5-7% water. Its molecular weight is 892.5 and its probable constitution is as follows:



It is insoluble in alcohol and other organic solvents but exceedingly soluble in cold water; 40% (w/v) solutions are stable when neutral or slightly alkaline, and a 60% solution may be autoclaved; 10% solutions are isosmotic, but 20% and even 40% solutions are well tolerated intramuscularly.

## ESTIMATION

Sulphetrone may be estimated in blood, urine, cerebrospinal fluid, and tissues by diazotisation and coupling to N-(1-naphthyl)-ethylenediamine hydrochloride (Bratton and Marshall 1939), the resultant dye being measured colorimetrically or, better, absorptiometrically. Since sulphetrone mixes intimately with precipitated proteins, the conditions governing its optimal recovery were determined experimentally. They are critical and must be rigidly respected to obtain 90% recoveries of sulphetrone:

To 5 ml. of N/1 hydrochloric acid 0.5 ml. of blood or other body fluid is added and mixed well; 2.0 ml. of 12% (w/v) trichloracetic acid is added, and the well-mixed solution is filtered immediately through a No. 5 Whatman paper and repassed until brilliant; 3 ml. of filtrate is mixed with 0.05 ml. of 0.3% fresh (weekly) sodium nitrite and left for 3 min.; 0.05 ml. of 1.5% ammonium sulphamate is now added, and the solution left for 2 min. Finally 0.05 ml. of 0.1% N-(1-naphthyl)-ethylenediamine hydrochloride is added and mixed well.

The colour is allowed to develop for 30 min. before being read colorimetrically, or absorptiometrically with a Wratten 61 filter. All estimates of sulphetrone are given in terms of the anhydrous compound. By collaboration with the Tintometer Ltd., a standard Lovibond colour disc is available for the rapid reading of blood and body-fluid estimates falling within the range of 0-9 mg. per 100 ml.

It has been shown (Brownlee *et al.* 1948) that the visible absorption spectra of the naphthyl-ethylene-diamine derivatives of diaminodiphenylsulphone and of sulphetrone are substantially different in preparations containing equivalent amounts of diaminodiphenylsulphone.

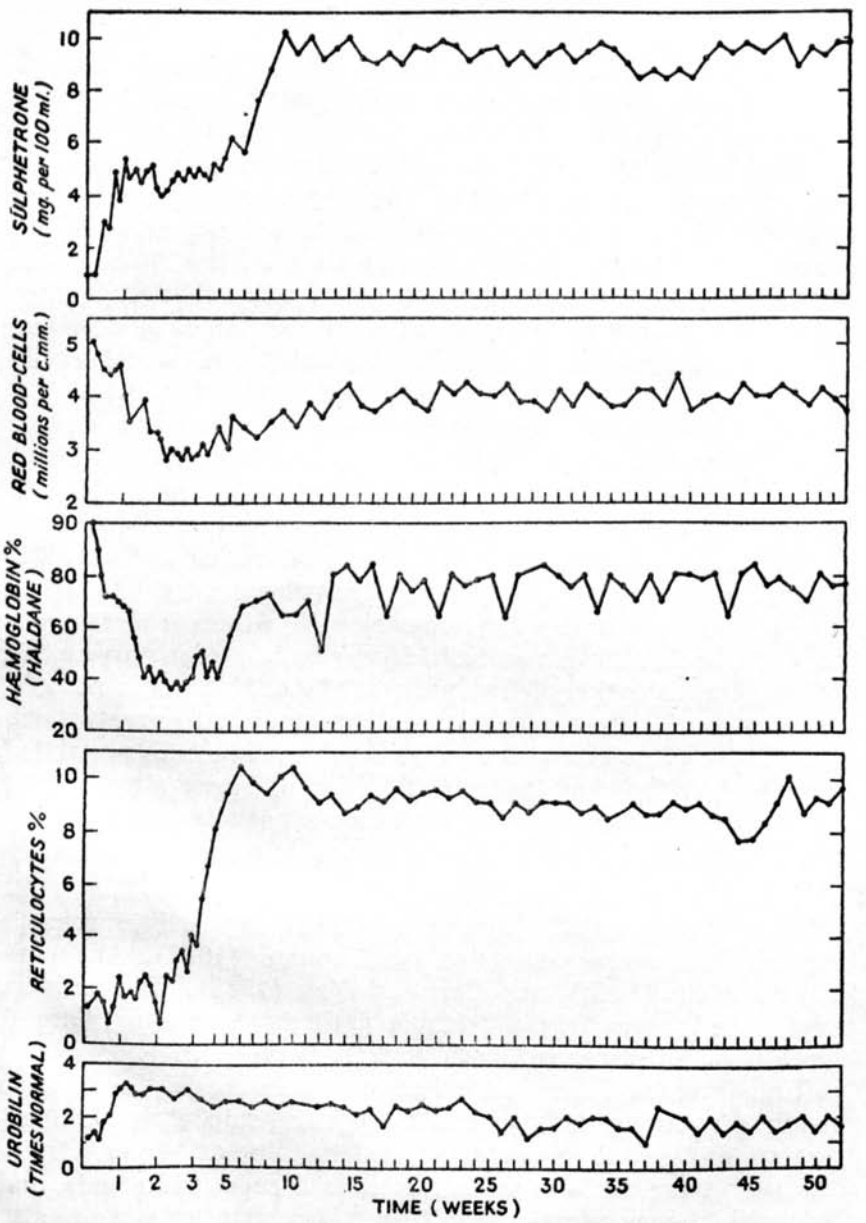
## TOXICITY

The *acute toxicity* of sulphetrone when given by mouth experimentally to animals is so slight that it cannot be determined with any certainty (Brownlee *et al.* 1948). Comparison of blood levels at death between ordinary sulphonamides and sulphetrone show the latter to be many times less acutely toxic than sulphanilamide. In the dog acute toxic symptoms are absent with blood-sulphetrone levels as high as 160 mg. per 100 ml. This fact, together with the observation that conjugated derivatives cannot be detected in blood or urine, is a strong link in the chain of evidence that sulphetrone is not easily hydrolysed. Acute toxic effects are not observed in man.

*Chronic toxicity.*—Very large doses of sulphetrone can be given by mouth for a long time to mice and dogs without producing ill effects. In rabbits, however, a diet containing 4% sulphetrone causes blood changes: (1) a slight but continuous hemolytic anemia marked by a concurrent reticulocytosis; (2) a progressive anemia due to lack of iron owing to competition for alimentary iron by sulphetrone, with which iron forms a non-absorbable complex, and which may be treated, or prevented, by the administration of iron parenterally or by mouth; and (3) an anemia of slower development but precipitate onset which seems to be of nutritional origin, since it may be both prevented and cured by the simultaneous exhibition of brewers' yeast, and is probably caused by the limitation and alteration of the bacterial flora of the gut by sulphetrone (Brownlee and Tonkin 1941).

That an essentially similar condition develops in man is implicit in the cases reported by Madigan (1948), Anderson and Strachan (1948), and Clay and Clay (1948). The problem was studied intensively in 2 female patients presenting bilateral fibrocaseous disease, and both at first gravely ill.

Sulphetrone-balance experiments were made for twelve months, involving daily estimates of blood-sulphetrone concentrations and of the amounts excreted in urine and feces. Daily estimates were made of reticulocytes, red cells, white cells, and hemoglobin concentrations; differential cell counts were also made. In all, estimates were made on 298 occasions. The urine was examined for urobilin (Watson 1936) and abnormal pigment; total porphyrin estimates were made weekly on 24-hour specimens, subsequently to be abandoned because the estimates fell within normal limits. Reference is made below to a chemical study of metabolic products of sulphetrone.




The information collected has allowed the study of several related problems also to be discussed. The figure summarises the typical response of a patient to sulphetrone by mouth; the dose was at first 3 g. daily (0.5 g. every four hours) and thereafter raised at seven-day intervals by increments of 1 g. until a therapeutic blood level of 7.5-10 mg. per 100 ml. was reached.

The weekly estimates are means of seven daily observations. As with the rabbit, the residual effect is a hemolytic anemia, which stimulates reticulocytosis. The red cells were crenated, and hemolysis in fragility tests was observed at 0.45 and increased at 0.48% NaCl.

In all the 94 cases examined no distinguishable effects on the numbers or distribution of white cells were observed. Consistent with the reticulocytosis observed in man there was an increase in the primitive red cells of the bone-marrow.

The range within which the differential red bone-marrow counts fell in 11 tuberculous patients treated with sulphetrone was as follows:

| | | Per cent | | | Per cent |
|---|---|---|---|---|---|
| A | Polymorphs | 3.5 – 34.4 | | Lymphocytes | 3.8 – 22.8 |
| | Metamyelocytes | 9.0 – 33.4 | | Monocytes | 0.4 – 10.8 |
| | Myelocytes | 1.8 – 11.5 | | Plasma cells | 0.2 – 2.6 |
| | Premyelocytes | 1.2 – 4.0 | B | Normoblasts | 24.0 – 37.5 |
| | Myeloblasts | 1.2 – 4.0 | | Erythroblasts | 0.4 – 1.0 |

The proportion of cells of the myeloid series (A) to nucleated red cells (B) is 1.24—2.5 to 1. This falls within the limits for normal marrow counts (Young and Osgood 1935). Estimates made on 2 of the 11 patients before treatment started gave ratios of 6.4 to 1 and 9.25 to 1.

*Abnormal blood pigments.*—The blood of animals and man containing therapeutic concentrations of sulphetrone is usually dark brown, and the pigment is restricted to the red cells, even though sulphetrone is distributed within plasma and cells. Examined spectrometrically (Beckman) the blood may show no specific absorption characteristic of known abnormal blood pigments. In a proportion of cases, which maybe as high as 20%, the specific absorptions of methemoglobin (630 m $\mu$) may be detected. Sulphemoglobin (618 m $\mu$) is absent. Methemoglobin may be identified visibly by spectroscope but only in the strongest solutions of laked blood which will just transmit light. When it is present, calculations from the extraction coefficients of the wave-length corresponding to oxyhemoglobin (540 m $\mu$) and methemoglobin (630 m $\mu$) suggest that the amount present is about 4-12% of the total hemoglobin.

Blood containing concentrations of sulphetrone above 10 mg. per 100 ml. may contain additional abnormal blood pigments. For example, of 904 spectrometric estimates in this category 23 were abnormal: 18 contained sulphemoglobin, and 5 appreciable amounts of methemoglobin. In all other cases in which

sulphemoglobinemia was observed in patients with blood-sulphetrone levels below 10 mg. per 100 ml. other drugs were implicated, phenacetin five times and methylacetanilide once. An additional abnormal pigment may be identified by a generalised absorption in the area 600-620 m $\mu$. This may be a hemoglobin-sulphetrone complex or a hemoglobin-sulphetrone degradation product.

*Oxygen capacity of the blood.*—Whatever the precise nature of the abnormal pigment, its ultimate importance depends on whether or not there is variation in the oxygen-carrying capacity of the blood. A series of 8 patients, with blood-sulphetrone levels of 7.4-9.2 mg. per 100 ml., were observed for ten weeks in which five repeated estimates were made of the oxygen-capacity of blood, together with spectrometric estimates. The analyses were made in the constant-volume Van Slyke apparatus. In this small series the estimates of oxygen-capacity were lower than normal in 5 cases, though associated with the presence of methemoglobin in only 2 (Table 1). The rate of oxygen (air) uptake was always slower than in normal blood.

TABLE 1.—*Oxygen-capacity of blood of 8 tuberculous patients treated with sulphetrone. Two normal estimates are included. The presence or absence of methemoglobinemia is indicated.*

| Case No. and sex | Oxygen found (vols. %) | Hb % (Haldane) | Oxygen calculated from Haldane value (vols. %) | Methemoglobinemia (spectrometric) |
|---|---|---|---|---|
| Normal: | | | | |
| 1 (F) | 15.3 | 82 | 18.6 | 0 |
| 2 (F) | 16.4 | 90 | 18.3 | 0 |
| Tuberculous: | | | | |
| 3 (F) | 12.0 | 86 | 14.0 | + |
| 4 (M) | 10.6 | 76 | 13.5 | + |
| 5 (F) | 16.3 | 90 | 18.1 | 0 |
| 6 (M) | 16.3 | 86 | 18.8 | 0 |
| 7 (M) | 14.5 | 94 | 15.4 | 0 |
| 8 (M) | 16.7 | 88 | 18.8 | 0 |
| 9 (M) | 14.8 | 88 | 16.6 | 0 |
| 10 (M) | 12.8 | 92 | 13.8 | 0 |

*Blue coloration of patients.*—Associated with these facts is the clinical observation that many patients treated with sulphetrone show a leaden-blue coloration of skin and mucosae. The tint and distribution of the colour are reminiscent of sulphemoglobinemia, which, as noted above, is absent. The intensity of pigmentation is not related to the blood-sulphetrone level but is greatest during the early period of adjustment. It is less obvious in the young, and most pronounced in those with low vital capacity. There is at the same time a quantity of violet-blue dye in the urine. Clinically the condition does not contra-indicate the exhibition of sulphetrone.

*Specific toxic effects.*—Sulphetrone shares with the sulphonamides the property of causing hypermia and hyperplasia of the thyroid gland in laboratory animals. The hyperplasia is thought to result from diminished synthesis of thyroid hormone. The tests showed sulphetrone to be the least and sulphaguanidine the most toxic, with sulphadiazine intermediate. The effect has not been observed in man.

*Effect on alkali reserve.*—The lack of acute toxicity and the low chronic toxicity, with freedom from the characteristic nervous sequelae seen in experiments on animals after the administration of diaminodiphenylsulphone, make it clear that sulphetrone is not degraded to any extent to diaminodiphenylsulphone in the body.

A point which must not be overlooked is the possible hydrolysis of one or more of the four sulphonated side-chains, and animal experiments were made to study this point (Brownlee *et al.* 1948). Simple hydrolysis with liberation of sodium acid sulphate appeared likely, but experiments on rabbits given sulphetrone by mouth and parenterally, and on dogs given sulphetrone parenterally, showed increased alkali in the blood. Comparison with the effects of administering alkali parenterally showed the increase in alkali reserve to be consistent with the liberation of one molecule of sodium hydroxide from each molecule of sulphetrone. This would involve the production of a condensation product of sulphetrone, and there is experimental evidence in man pointing to the excretion of a colored complex of this kind in the urine.

It is significant that tests made on rabbits given sulphetrone for long periods showed the animals to be capable of establishing an equilibrium in plasma-alkali balance.

The alkali reserves of untreated and treated tuberculous patients are compared in Table 2. In the first column are the estimates of alkali reserve after seven days' treatment; in the second, two months later. Included within the second series are 3 patients who had received sulphetrone for seven days only. The estimates were made within 30 min. from freshly drawn

TABLE 2.—*Effect of sulphetrone in plasma $CO_2$-combining capacity, in volumes % in man.*

| Case No. | Oct. 8, 1947 | | Dec. 8, 1947 | | Dec. 8, 1947 | |
|---|---|---|---|---|---|---|
| | Blood-sulphe-trone (mg. per) 100 ml.) | Carbon-dioxide capacity of plasma (vols. %) | Blood-sulphe-trone (mg. per 100 ml.) | Carbon-dioxide capacity of plasma (vols. %) | Normal patient | Carbon-dioxide capacity of plasma (vols. %) |
| 1 | 3 | 61.7 | 7.5 | 64.3 | A | 64.8 |
| 2 | 5 | 61.1 | 8.1 | 63.0 | B | 65.5 |
| 3 | 4 | 63.6 | 5.3 | 64.9 | C | 58.0 |
| 4 | 4 | 63.0 | 7.2 | 58.6 | D | 65.5 |
| 5 | 4 | 53.0 | 2.0* | 58.6 | E | 57.2 |
| 6 | ------ | ------ | 4.0 | 55.4 | F | 63.9 |
| 7 | ------ | ------ | 4.1 | 57.3 | ------ | ------ |
| 8 | ------ | ------ | 3.2 | 56.7 | ------ | ------ |

*/Drug being withdrawn.

blood centrifuged under paraffin; the $CO_2$-capacity was determined by the volumetric method of Van Slyke. It will be seen that there is no significant change in the $CO_2$-combining capacity of the plasma after protracted treatment with sulphetrone.

Sulphetrone is well tolerated if the exhibition of the drug is gradual. Nevertheless, during this period of adjustment mild symptoms of toxicity are seen (Madigan 1948)—slight nausea, mild headache, and difficulty in reading. These may be associated with the physiological accommodation the patient is called on to make in alkali reserve. The symptoms are relieved best by sodium bicarbonate and not so well by lactate or citrate. The bicarbonate ion ($HCO_3$)—may therefore be involved.

## ABSORPTION AND EXCRETION

Experiments on mice and dogs in which single small or large doses were given showed that, after a certain point is reached, large increases in dose do not lead to either a higher blood-sulphetrone level or increased absorption. Similar observations have been made with the more complex sulphonamides at somewhat higher doses. The rabbit is anomalous, for increase in dosage increases the absorption of sulphetrone and its concentration in the blood. In dogs the same total amount of sulphe-

trone given in divided doses at intervals of a few hours led to a higher blood-sulphetrone level than when given as a single dose. A similar effect was seen in man in ten experiments restricted to 96 hours. The results of an experiment on dogs in which sulphetrone was injected first into the large and then into the small intestine, the ileocecal junction having been tied, support the view that absorption is largely confined to the small intestine. It follows that optimal conditions of absorption are likely to follow uniformly divided doses.

*Sulphetrone and metabolism products in urine and feces.*— Two female tuberculous patients given sulphetrone were the subject of drug-balance experiments during a continuous period of twelve months. The intake of sulphetrone averaged 6.5 g. daily; the amounts of sulphetrone in urine and feces were estimated. As already indicated, the recovery of sulphetrone from urine was good, 90-95%, but only 75-82% could be obtained from feces. Sulphetrone in the feces does not indicate only the amounts which remain unabsorbed, since some secretion takes place into the bile and from the ileum. It is impossible to relate the amount of sulphetrone in the urine to the amount absorbed, for still other channels of excretion are open to it. After the initial period of adjustment, as little as two-fifths, and later as much as three-fifths, passes through the kidneys; as much as two-fifths and as little as a fifth was recovered from the feces.

The urine of animals and man receiving sulphetrone contains a diazotisable substance which, when coupled with naphthylethylenediamine, forms a dye with an absorption similar to that of sulphetrone. Heating with N/1 hydrochloric acid for 30 min. does not increase the amount of diazotisable material; thus there is probably no conjugation. The concentration of sulphetrone in urine is high—usually 10 to 20 times that in the blood.

The urine of patients treated with sulphetrone is a dark smoky-brown. The pigment may be separated chromatographically on a column of activated alumina (Brockmann: Messrs. Savory and Moore).

Most of this pigment separates as a dark brown ½ in. band at the top. After washing with a similar volume of distilled water about 95-97% of the total diazotisable material passes through the column. The pigment may now be developed with 0.5% hydrochloric acid to give a blue-violet narrow band, followed closely by a mauve-pink broader band, with brown (bile) pigments left behind. The violet-blue pigment is soluble in 0.5% hydrochloric acid and, diazotised and coupled, appears to account for about 2% of the total sulphetrone in the urine. The mauve-pink solution in hydrochloric acid couples with difficulty; calculated as sulphetrone it appears to account for about 0.5% of the total.

It is interesting to speculate on the part played by these dyestuffs in the leaden-blue coloration of sulphetrone-treated patients excreting these dyes in their urine. The dyes appear to be condensation products of two or more molecules of sulphetrone, which, it will be recalled, loses alkali in the blood-stream, probably by just such a condensation procedure.

*Renal clearance.*—Sulphetrone is excreted by the kidney very fast. In rabbits the clearance was 58% of that of creatinine, or two or three times as fast as that of sulphanilamide, while in the dog the clearance was five times as fast as that of sulphanilamide (Brownlee *et al.* 1948).

The opportunity arose in a patient, repeatedly catheterised for the diagnosis of renal tuberculosis, to determine the clearance-rate of sulphetrone by direct comparison with sulphanilamide. Both drugs were given intravenously in a dose of 0.05 g. per kg. and five estimates were made at intervals of 30 min. The mean clearance for sulphanilamide $\pm$ S.D. was 12.2 $\pm$ 2.4, whereas for sulphetrone, tested after an interval of eight days, it was 62.1 $\pm$ 11.3.

It seems that, in man, tubular resorption of sulphetrone is small, and that the rate of clearance is about four times as fast as with sulphanilamide.

## DISTRIBUTION OF SULPHETRONE IN TISSUE

Sulphetrone rapidly penetrates all tissues except brain, but it appears in cerebrospinal fluid more slowly than do sulphonamides. The tissues of animals show similar concentrations whether the sulphetrone is given in a sufficient dose intravenously or by mouth over a period of ten weeks (Brownlee *et al.* 1948). In experiments on animals concentrations of sulphetrone in liver, kidney, and spleen are always greater than in plasma; the same is true for man (Table 3). The relation between absorbed sulphetrone, sulphetrone in transport, and sulphetrone in kidney clearance does not seem to be a simple one, and conditions other than the concentration of sulphetrone in the plasma appear to cooperate in determining the amount in the tissues. Plasma-sulphetrone levels obtained in nephrectomised rabbits are many times those seen in normal rabbits, and many times the level attained in other tissues. The concentrations in the bile of both normal and nephrectomised animals are very high; in the normal animal the concentration in bile is about twelve times the plasma value and must be considered to be sulphetrone in transport, so that biliary excretion makes a significant contribution to the clearance of the drug.

TABLE 3.—*Penetration of sulphetrone into various tissues in man, expressed as mg. per 100 ml. of blood or other fluid and as mg. per 100 g. of tissue.*

| Tissues | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 |
|---|---|---|---|---|---|
| Blood | 12.4 | 4.1 | ------ | 22.4 | 17.7 |
| Plasma | 8.1 | 2.2 | ------ | 13.3 | 10.3 |
| Corpuscles | 4.3 | 1.9 | ------ | 9.1 | 7.4 |
| Cisternal fluid | 1.8 | 1.3 | 1.6 | 3.7 | 2.9 |
| Bile | 140.0 | 19.9 | 130.0 | 254.0 | 171.4 |
| Liver | 14.0 | 11.5 | 3.7 | 30.0 | 29.4 |
| Kidney | 17.6 | 20.2 | 23.7 | 97.5 | 73.1 |
| Spleen | 30.5 | 9.8 | 2.4 | 40.0 | 46.0 |
| Normal lungs | 10.4 | 7.4 | 4.9 | 37.0 | 27.2 |
| Caseous lungs | ------ | ------ | 7.3 | ------ | 44.2 |
| Bone-marrow | ------ | 0.0 | 0.2 | ------ | 0.4 |
| Heart muscle | 14.0 | 6.0 | ------ | ------ | 19.2 |
| Striated muscle of thigh | ------ | 17.8 | ------ | 42.7 | 28.0 |
| Ileum | 21.0 | ------ | 22.7 | ------ | 22.1 |
| Cerebral hemispheres | ------ | 5.9 | 2.0 | 6.1 | 3.7 |
| Peritoneal fluid | 66.0 | ------ | ------ | ------ | 27.4 |
| Pericardial fluid | 6.3 | ------ | ------ | ------ | 8.2 |
| Mediastinal glands | ------ | 5.6 | ------ | ------ | ------ |
| Pus from mediastinal gland | ------ | 16.0 | ------ | ------ | ------ |

The study of the distribution of sulphetrone in the tissues in man has necessarily been confined to necropsy specimens. Cases 1, 2, and 3 of Table 3 show the distribution of sulphetrone in different tissues when the blood-sulphetrone was at a therapeutic level, at death after tuberculous meningitis (2 cases) and hemoptysis (1 case). Cases 4 and 5 show the distribution of sulphetrone in different tissues after excessively high and long-maintained blood-sulphetrone levels caused by constipation allowing sulphetrone to accumulate in the small intestine. In both cases the blood-levels were maintained at 17.5 mg. per 100 ml. for more than five days. Death from anoxia followed from the inability of the red cells to transport oxygen.

SUMMARY

The chemical and physical properties of sulphetrone and its estimation in body fluids are described.

The acute toxicity of sulphetrone in experiments on animals is very slight. No acute toxic effects have been observed in man.

The chronic hematotoxic effects in rabbits and in man include a hemolytic anemia, an anemia of iron lack, and an anemia of nutritional origin.

Sulphetrone has a goitrogenic effect similar to that of sulphonamides, but this is only slight in experiments on animals and has not been observed in patients receiving the drug.

Sulphetrone given by mouth or parenterally raises the alkali reserve of the plasma of rabbit and dog. The probable mechanism is discussed. Given over a long period it brings about some tolerance. It is thought that the period of adjustment seen during the first three weeks of sulphetrone therapy is associated with adjustments of alkali reserve. The minor discomforts encountered during this period are relieved by bicarbonate, but not so well by other alkalis.

Though very soluble in water, sulphetrone is slowly absorbed from the intestinal tract—most from the small intestine, little from the large.

Sulphetrone is cleared by the kidney very quickly, and fluid must be limited to maintain blood-sulphetrone concentrations; there is also a substantial excretion into the bile and to a lesser extent into the ileum. The drug is not conjugated; so there is no danger of crystaluria.

Sulphetrone penetrates all tissues, except brain, very rapidly. It passes into the cerebrospinal fluid more slowly than do sulphonamides.

Thanks are due to Dr. A. I. Ross, of Farnborough County Hospital, who was responsible for the bone-marrow biopsies; Dr. T. Anderson and Dr. D. G. Madigan for permission to draw on their clinical necropsy findings; Dr. E. I. Short for some of the oxygen-capacity and spectrometric observations; Dr. C. L. Oakley for the histological reports.

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