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THE EFFECT OF PAS ON THE DISTRIBUTION OF SULPHETRONE AFTER SIMULTANEOUS ADMINISTRATION OF THE DRUGS

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During experiments on the combined use of PAS and sulphetrone in mice, blood concentrations of sulphetrone after oral administration of the two drugs together were higher than after sulphetrone alone. A study of this effect, and of its bearing on the toxicity of sulphetrone, has been made in mice, rabbits, and dogs, and possible explanations have been considered.

It seemed that the most probable ways in which the PAS could cause more sulphetrone to be present in the blood stream were:

1. By increasing absorption from the gut.
2. By reducing excretion of the drug.
3. By diminishing the affinity of the drug for protein and preventing its storage in the body.

These points have been investigated by administering sulphetrone alone and with PAS, and comparing the blood concentrations after oral and parenteral administration, the urinary and biliary excretion, the distribution in the tissues, and the degree of binding to serum proteins *in vitro*.

METHODS

Estimation of the Drugs

Sulphetrone was estimated by a modification of the method introduced by Bratton and Marshall (1939) for the estimation of sulphonamides. An aliquot of a suitably prepared blood filtrate, tissue extract, or urine dilution is diazotized by the addition of sodium nitrite, and the resulting diazonium compound is coupled with *N*-(1-naphthyl)-ethylenediamine hydrochloride after destruction of the excess nitrite with ammonium sulphamate. Sulphetrone tends to be adsorbed by denatured protein, and the optimum conditions determined experimentally by Brownlee, Green, and Woodbine (1948) must be rigidly adhered to. Since the method is dependent on the diazonium reaction of free aromatic amino groups, it does not distinguish between the intact molecule, hydrolytic products—such as diaminodiphenylsulphone and "semi-sulphetrone" (4-γ-phenyl-*n*-propylamino - 4' - aminodiphenylsulphone-$\alpha\gamma$-disodium sulphonate)—or other metabolites containing an amino group. The drug concentrations given include such metabolites, expressed in terms of sulphetrone.

The two drugs were estimated in the presence of each other by determining the total concentration, in terms of sulphetrone, by the diazonium reaction and the amount of PAS by the reaction with *p*-dimethylaminobenzaldehyde. A correction for the contribution of PAS to the total diazonium reaction can then be calculated and the sulphetrone concentration obtained by difference (Short, 1951). That PAS does not affect the amount of colour produced by sulphetrone in the diazonium method and that sulphetrone does not affect the estimation of PAS by *p*-dimethylaminobenzaldehyde is shown by the excellent recoveries of both drugs obtained from *in vitro* estimations (Short, 1951). Thus there is little doubt that the raised sulphetrone blood concentration observed when the two drugs are given together is not due to some anomaly in the estimations but to some effect of the PAS *in vivo*.

Throughout the investigation PAS was used as the sodium salt, but doses and concentrations are expressed in terms of the free acid.

Determination of Blood Concentrations

Mice.—Groups of 18 mice each weighing about 20 g. were used. The drugs were administered orally by stomach tube, as suspensions in acacia, in doses of 0.625 g./kg. of each drug, and parenterally as solutions in doses of 0.25 g./kg. The blood concentration in pooled tail blood was determined at 0, $\frac{1}{2}$, 1, 2, 3, $4\frac{1}{2}$, and 6 hr.

Rabbits.—Young male rabbits, weighing 2–3 kg., were given the drugs in suspension or solution by stomach tube or parenterally, in doses of 0.5 g./kg. of each drug. Concentrations were determined at intervals on blood from the marginal ear vein.

Dogs.—Blood concentration-time curves were constructed from values in seven dogs after oral administration of the drugs by stomach tube. The dogs were first given sulphetrone (0.2 g./kg.), and then, after two weeks, PAS and sulphetrone, each in doses of 0.2 g./kg. Blood was withdrawn from the saphenous vein at 0, $\frac{1}{2}$, 1, 2, 3, $4\frac{1}{2}$, 6, and 24 hr. and analysed.

Similar cross-over tests on two dogs were used to determine blood concentrations after parenteral admin-

istration. The animals were first given sulphetrone (0.2 g./kg.) by intravenous or intraperitoneal injection of the aqueous solution, and blood concentrations were determined at intervals. After two weeks the combined drugs were administered, PAS (0.2 g./kg.) by stomach tube and, 10 min. later, sulphetrone (0.2 g./kg.) intravenously or intraperitoneally. Blood concentrations were determined as before. Because of its sparing solubility, it was not practicable to administer PAS parenterally at the doses employed, but—since it appears in the blood stream within 5 min. of oral administration and since peak levels are rapidly reached ($\frac{1}{2}$–1 hr.)—a similar effect was obtained by giving it, orally, shortly before the parenteral dose of sulphetrone.

Determination of Excretion Rate

Urinary excretion was determined in rabbits and dogs. Because of the fairly wide variation in individual animals, cross-over tests on pairs of animals were used, the animals which were given sulphetrone alone being given combined sulphetrone and PAS a fortnight later, and vice versa. The doses used were the same as those for the determination of blood concentrations; the animals were hydrated before the experiment with 25 ml. water/kg. (rabbits) or 10 ml./kg. (dogs). Urine was withdrawn by catheter at 0, 1, 2, 4, 6, and 24 hr. and analysed.

Biliary excretion was measured in dogs (0.2 g./kg. of each drug) and rabbits (0.5 g./kg. of each drug) by cannulating the common bile duct under pentobarbitone anaesthesia. The bile was collected and its drug content estimated hourly for 6 hr.

Concentrations of the Drugs in Tissues

To study the distribution of the drugs in tissues, groups of six mice were given 0.625 g./kg. of sulphetrone or 0.625 g./kg. each of PAS and sulphetrone by mouth or 0.25 g./kg. by subcutaneous or intraperitoneal injection. The animals were killed 6 hr. after dosing, the tissues removed, pooled, and weighed, and the amount of drug estimated in lung, liver, kidney, spleen, heart, brain, gastro-intestinal tract, muscle, bone, and skin.

Binding of the Drugs to Serum Proteins

The most satisfactory method of studying the protein binding *in vitro* was a modification of the ultrafiltration method of Boxer, Jelinek, and Edison (1949). Possibly because it is present in solution as colloidal aggregates, sulphetrone does not pass through an ordinary cellophane membrane. Experiments with artificial membranes of varying pore-size, prepared from polyvinyl acetate or collodion, showed that the most satisfactory one was that obtained with a solution of 10% collodion in ether. The bags were prepared by pouring the solution into a 1.5 × 5 cm. tube and pouring it out again so that a thin film was left over the inside walls. The tube was rolled continuously for 5 min., to spread the film evenly and to allow the ether to evaporate. It was then filled with cold water to set the collodion film and to fix the pore size. After 5 min. the water was poured off and the membrane allowed to become almost dry before removing it from the tube. 10 ml. of a solution of the drugs in water or serum was placed inside the bag, which was then sealed by twisting the top and applying a pair of hot forceps. The bag was placed inside a 3 cm. centrifuge tube containing 10 ml. water, centrifuged for 10 min. and the concentration of drug inside and outside the bag determined. A correction for the volume of water passing through the bag was made by running a blank experiment with 10 ml. of water or serum in the bag and 10 ml. of water outside and measuring the increase in volume of the water in the centrifuge tube. The strengths of the solutions used were: sulphetrone 10 mg./100 ml. and PAS 20 mg./100 ml. together or separately.

Results and Discussion

Blood Concentrations

Sulphetrone is only slowly absorbed after oral administration; it appears in the blood stream in fairly low concentration within half an hour and is maintained at about the same concentration for at least 6 hr. Small amounts are still present after 24 hr. A typical mean blood concentration-time curve after oral administration of 0.625 g./kg. to mice is given in Fig. 1.

When PAS, in equal dosage, was administered simultaneously, a three- to four-fold increase in sulphetrone concentration was obtained within half to one hour; the concentration then gradually fell until at 6 hr. it differed little from that produced by the same dose of sulphetrone alone. This increase in sulphetrone concentration closely parallels the PAS concentration in the blood (Fig. 2).

Similar results were obtained in rabbits (Fig. 3) and in dogs (Fig. 5). The highest increase in concentration again corresponded approximately to the highest PAS blood concentration (Figs. 4 and 6). The combined administration of the two drugs had little effect on PAS blood concentrations. The effect on the sulphetrone concentration lasts longer in dogs than in mice and rabbits. This may be because the PAS concentration falls less rapidly in dogs (Fig. 6).

Typical sulphetrone blood concentration-time curves after intraperitoneal and subcutaneous injection of 0.25 g./kg. of the drug to mice are also included in Fig. 1. Peak concentrations were obtained at $\frac{1}{2}$ hr. with a fairly rapid fall in 2 to 3 hr. followed by a more gradual drop to 6 hr., when only a very low concentration remained. Again, higher concentrations were obtained when an equal dose of PAS was given with the sulphetrone, but the effect was less than when the drugs were given orally, the maximum sulphetrone concentration being only one and a half to twice that obtained when sulphetrone was given alone. The effect lasted for about three hours and closely paralleled the PAS blood concentration (Fig. 2).

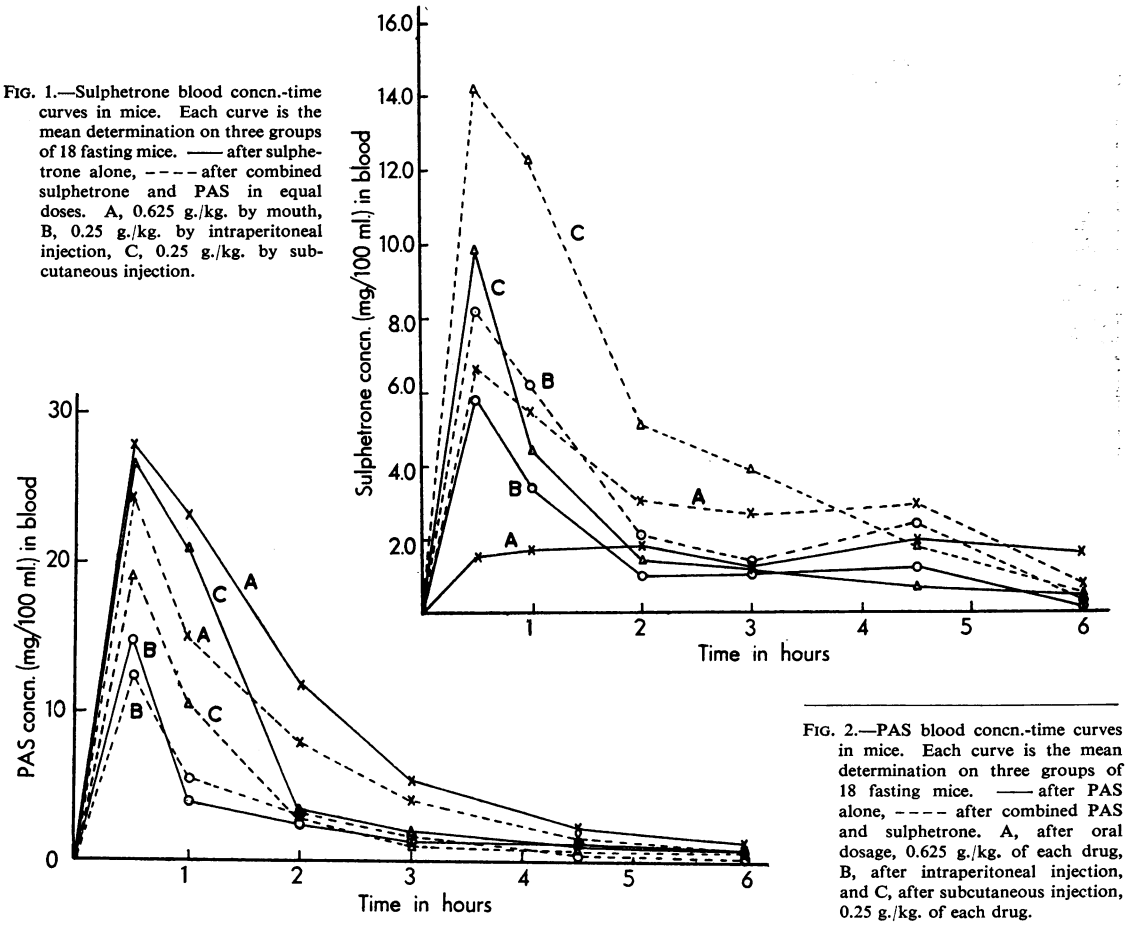

FIG. 1.—Sulphetrone blood concn.-time curves in mice. Each curve is the mean determination on three groups of 18 fasting mice. —— after sulphetrone alone, - - - - after combined sulphetrone and PAS in equal doses. A, 0.625 g./kg. by mouth, B, 0.25 g./kg. by intraperitoneal injection, C, 0.25 g./kg. by subcutaneous injection.

FIG. 2.—PAS blood concn.-time curves in mice. Each curve is the mean determination on three groups of 18 fasting mice. —— after PAS alone, - - - - after combined PAS and sulphetrone. A, after oral dosage, 0.625 g./kg. of each drug, B, after intraperitoneal injection, and C, after subcutaneous injection, 0.25 g./kg. of each drug.

In rabbits, at the dose employed (0.5 g./kg.), the effect was not so marked, possibly because of the high sulphetrone concentrations which were obtained initially. When this initial high concentration had fallen, however, increases of sulphetrone concentration of 15–25% after intraperitoneal injection and 25–30% after subcutaneous injection were maintained for 4½ hr. when the two drugs were given simultaneously. These concentrations corresponded with the PAS blood concentration, falling off as the PAS concentration decreased (Fig. 4).

In dogs the effect was studied after a PAS blood concentration had been established by giving the drug by mouth, and similar results were obtained after both intravenous and intraperitoneal injections of sulphetrone (0.2 g./kg.). Both methods of administration gave an increase of 20–40% in sulphetrone concentration over that after giving sulphetrone alone, and this was maintained for at least 6 hr. (Fig. 5). The concentration of sulphetrone at 24 hr., when the PAS blood concentration was insignificant, did not differ from that with sulphetrone alone.

The fact that the sulphetrone blood concentration is increased to a greater extent when the two drugs are given by mouth than when they are given parenterally indicates that some increased absorption from the gut must occur, although, since there is some effect with parenteral administration, this cannot be the complete explanation. Since the difference after oral administration is observed within half an hour, the increased absorption cannot result from delayed passage of the drug to the caecum. A possible explanation is that, because of its preferential combination with protein which is described later, PAS may free sulphetrone from combination with substances of high molecular weight within the intestine and thus make it available for absorption in larger amounts.

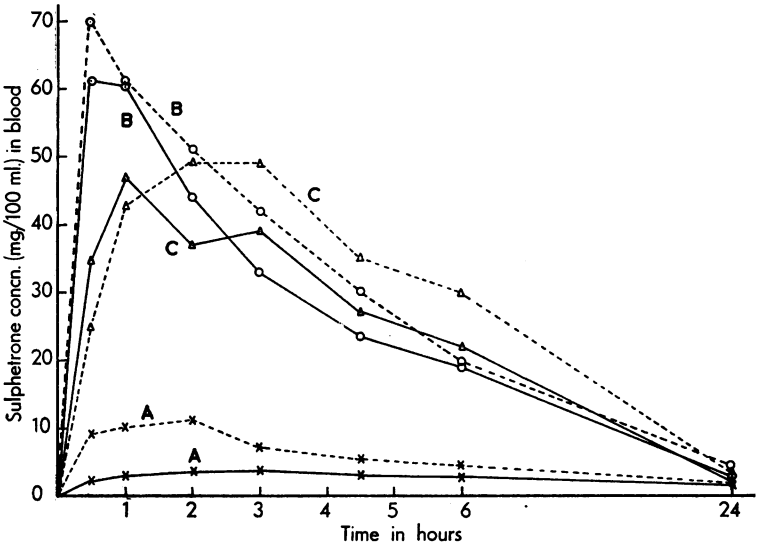

FIG. 3.—Sulphetrone blood concn.-time curves in rabbits. Each curve is the mean determination on six fasting rabbits. —— after sulphetrone alone, 0.5 g./kg., and - - - - after combined sulphetrone and PAS, 0.5 g./kg. of each drug. A, by mouth, B, by intraperitoneal injection, C, by subcutaneous injection.

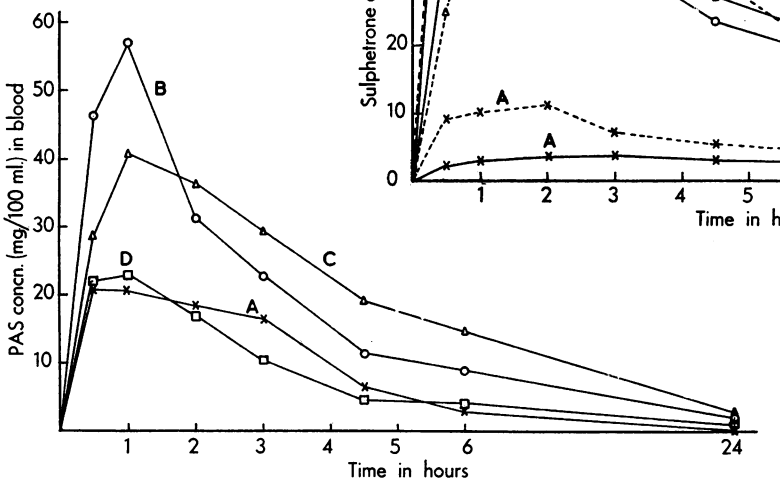

FIG. 4.—PAS blood concn.-time curves in rabbits. Each curve is the mean determination on 6 fasting rabbits. Dose: 0.5 g./kg. of each drug. A, combined PAS and sulphetrone by mouth, B, combined PAS and sulphetrone by intraperitoneal injection, and C, by subcutaneous injection, D, PAS alone by mouth.

Excretion of the Drugs

There was little difference in the total urinary excretion of sulphetrone in rabbits in the first 24 hr., whether the drug was given alone or with PAS, but the excretion at 1, 2, and 4 hr. was two and a half times greater when PAS was given (Fig. 7A), reflecting the higher blood concentrations which were obtained. In dogs, where the higher sulphetrone blood levels were maintained for a longer period, the increase in urinary excretion was more marked (Fig. 8A) and the total urinary excretion in the first 24 hr. was about four times greater when the two drugs were given together than when sulphetrone was given alone.

The urinary excretion after parenteral administration of the combined drugs also showed an increase corresponding to the higher blood levels. Increases of 25–30% after intraperitoneal and subcutaneous injection were found in rabbits (Fig. 7B and C) at times corresponding to similar increases in blood concentrations. In dogs, urinary excretion was increased by 20–40% after intraperitoneal injection (Fig. 8B), the differences at various periods during the first 24 hr. again being similar to the differences in blood concentrations. After intravenous injection of sulphetrone in dogs as much as 92% appeared in the urine in the first 24 hr., and, because of this high excretion rate, the effect of PAS on the urinary excretion was less clear-cut. That there was, however, some increase in sulphetrone excretion when the drug was injected after establishing a PAS blood concentration was apparent at periods during the first 24 hr., and the total excretion was increased to 96% of the dose (Fig. 8C).

Only small amounts of sulphetrone were excreted in the bile in rabbits and dogs, and this excretion was increased by simultaneous administration of PAS. Estimation of the hourly excretion in the bile for 6 hr. showed that a three- to four-fold increase in biliary excretion was consistently found in the rabbit (Fig. 7D) and that the effect was somewhat greater in the dog (Fig. 8D).

The consistent increase in excretion after combined administration of the drugs by all routes clearly establishes that the higher sulphetrone blood concentrations obtained cannot result from competitive renal elimination or blockage of any other excretion route since, if this occurred, the excretion

FIG. 5.—Sulphetrone blood concn.-time curves in dogs. ——after sulphetrone alone, - - - - after combined PAS and sulphetrone. Curves A are mean determinations on 7 dogs given first sulphetrone, 0.2 g./kg. by mouth, and then, after 2 weeks, PAS and sulphetrone. 0.2 g./kg. of each drug. Curves B and C represent similar cross-over tests, each on two dogs given 0.2 g./kg. sulphetrone first alone and then with 0.2 g./kg. PAS orally by intraperitoneal and intravenous injection respectively.

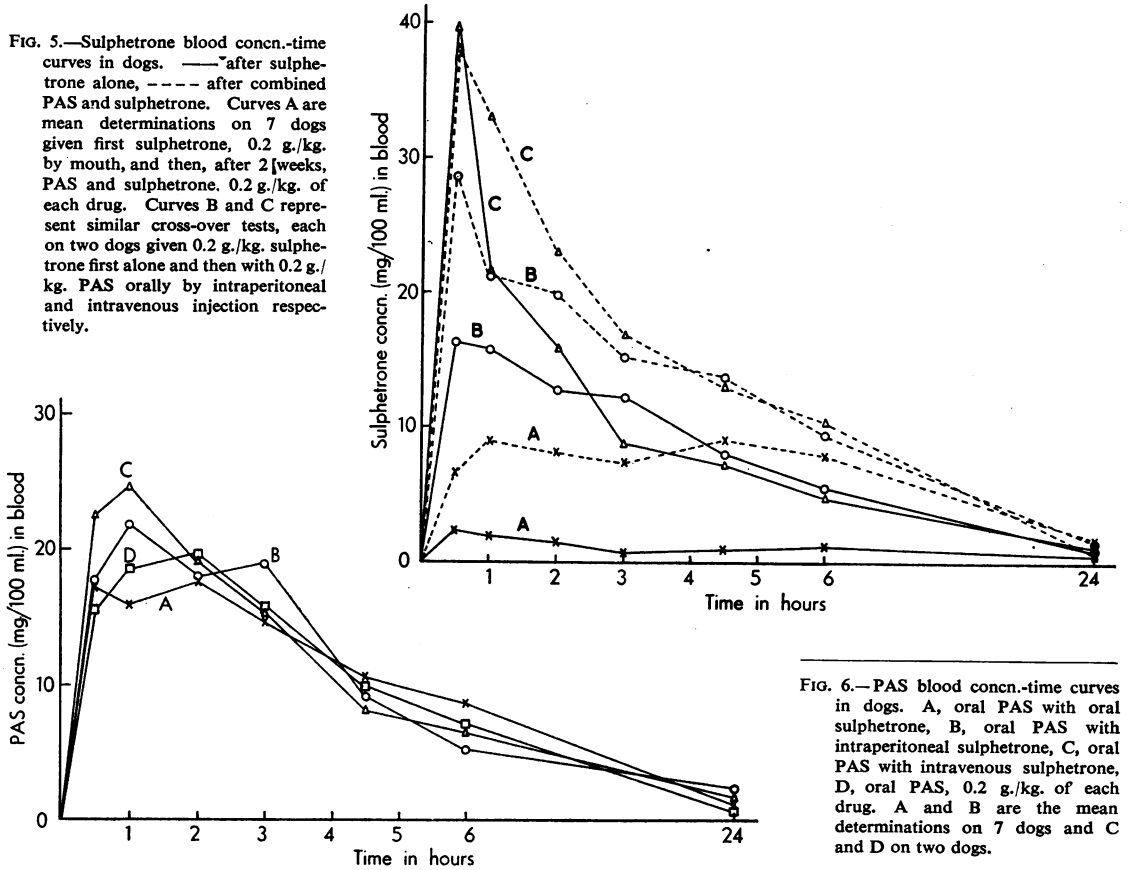

FIG. 6.—PAS blood concn.-time curves in dogs. A, oral PAS with oral sulphetrone, B, oral PAS with intraperitoneal sulphetrone, C, oral PAS with intravenous sulphetrone, D, oral PAS, 0.2 g./kg. of each drug. A and B are the mean determinations on 7 dogs and C and D on two dogs.

would decrease rather than increase. Furthermore, the differences in excretion rate correspond more or less closely with the differences in blood concentration, suggesting a corresponding increase in glomerular filtration rate.

Protein Binding and Tissue Distribution

Way, Smith, Howie, Weiss, and Swanson (1948) demonstrated the binding of PAS to plasma protein both *in vivo* and *in vitro*. Binding occurs to the extent of 50–60% with plasma concentrations of 4–10 mg./100 ml. *in vivo* and *in vitro*, and with 3.5% crystalline bovine albumin containing 5, 10, and 50 mg./100 ml. to the extent of 70, 60, and 50% respectively. The difficulties encountered in freeing sulphetrone from protein in the estimation of the drug suggest that it also has an affinity for protein and that it might be stored in organs by attachment to tissue protein. The increased sulphetrone blood concentration obtained when the two drugs are given together might then be due to a competitive action of the PAS, resulting in its preferential attachment to the tissue protein concerned with storage of the drugs. This possibility was studied by determining the *in vitro* serum binding of both drugs, separately and together, by ultrafiltration of solutions in horse serum through a collodion membrane and by estimating the concentration of the drugs in the tissues after oral and parenteral administration.

The results of the ultrafiltration experiments are given in Table I. For purposes of comparison, to

TABLE I

ULTRAFILTRATION OF SULPHETRONE (10 MG./100 ML.) AND PAS (20 MG./100 ML.) FROM HORSE SERUM AND WATER THROUGH A COLLODION MEMBRANE

(The results, expressed as percentages of theoretical amounts of drug in the ultrafiltrate when no protein binding occurs, are the means of six experiments in each case)

Drug Estimated	Solution in Bag	Mean Serum % in Ultrafiltrate ±S.E.	Mean Water % in Ultrafiltrate ±S.E.
Sulphetrone	Sulphetrone alone	12·9±0·33	101·6±0·31
" ..	Sulphetrone+PAS	25·2±0·90	103·2±0·90
PAS ..	PAS alone	14·4±0·32	102·0±0·34
" ..	PAS+sulphetrone	18·1±0·62	101·6±0·60

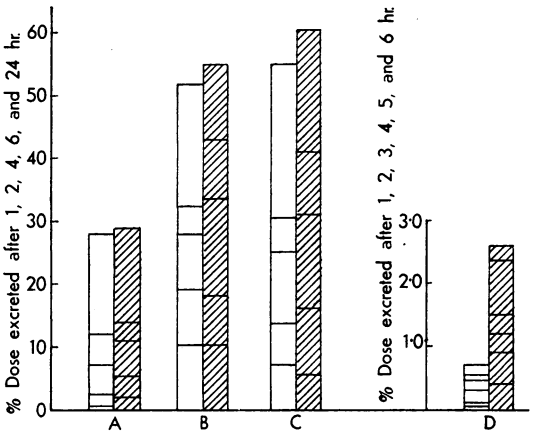

FIG. 7.—Excretion of sulphetrone in urine and bile in rabbits after sulphetrone alone 0.5 g./kg. (open) and combined sulphetrone and PAS, 0.5 g./kg. of each drug (cross-hatch). The urinary excretion is the mean 24-hr. excretion in cross-over tests on six rabbits. The biliary excretion is the mean 6-hr. excretion on three rabbits. Urinary excretion after: A, oral dosage, B, intraperitoneal injection, and C, subcutaneous injection. D, biliary excretion after oral dosage.

establish the accuracy of the method, and to ensure that the membrane was completely permeable to the drugs, parallel experiments using solutions in distilled water were performed simultaneously. Ultrafiltration through the membrane was complete when aqueous solutions were used, the theoretical amount of drug being present in the ultrafiltrate both from separate solutions of the drugs and the mixed solution, the possibility of adsorption of some of the drugs on to the membrane thus being excluded. Only 14.4% of the theoretical amount of PAS diffused through the membrane from a solution of the drug in horse serum, corresponding to 85.6% protein binding, a figure somewhat higher than that obtained by Way *et al.* using 3.5% bovine albumin. Protein binding of sulphetrone was found to occur to about the same extent: 12.9% of the theoretical amount of drug passed through the membrane, corresponding to 87.1% protein binding. A considerable increase in the amount of sulphetrone in the ultrafiltrate occurred with mixtures of the two drugs; the percentage of the theoretical figure passing through the membrane was double that obtained with sulphetrone alone. PAS showed a smaller but significant increase.

This reduced binding of PAS and sulphetrone when both are present suggests competition between the two drugs for a limited number of protein receptors with the balance in favour of PAS. Thus the increased sulphetrone blood concentrations obtained when the two drugs are given together could be explained by decreased formation of sulphetrone-protein complexes resulting in a reduction in the amount bound by the tissues. Confirmation was obtained from tissue distribution studies in mice.

Table II gives the distribution of sulphetrone in mice, 6 hr. after administration of the drugs. Very little difference was found between oral and parenteral dosage, except for the larger amounts present in the gastro-intestinal tract, presumably due to unabsorbed drug, in the former experiment. The results indicate that storage of the drug occurs mainly in the liver, since, apart from the gastro-intestinal tract, the highest concentration was obtained in the liver, with the kidney and spleen next, but with values only about half the liver concentration. Appreciable amounts were present in lung and skin, and only negligible amounts in the other organs. When the drug was given with PAS, a reduction in tissue concentrations occurred in accordance with the increased excretion. This decrease was most marked in the liver, the concentration differing little from that in the spleen and the kidney.

These results appear to establish that the increased sulphetrone blood concentrations obtained when

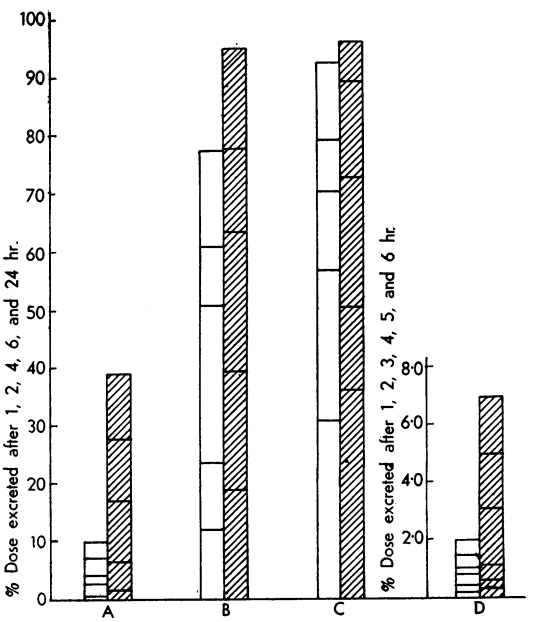

FIG. 8.—Excretion of sulphetrone in urine and bile in dogs after sulphetrone alone (open) and combined sulphetrone and PAS (cross-hatch). A represents the mean 24-hr. excretion in seven dogs given first sulphetrone, 0.2 g./kg. by mouth, and then, after 2 weeks, PAS and sulphetrone 0.2 g./kg. of each drug, B and C represent the mean 24-hr. urinary excretion in a similar cross-over test in two dogs given 0.2 g./kg. sulphetrone by intraperitoneal and intravenous injection respectively, first alone and then after 2 weeks, with 0.2 g./kg. oral PAS. D represents the mean 6-hr. excretion from the bile in two dogs after oral administration of 0.2 g./kg. of each drug.

TABLE II

DISTRIBUTION OF SULPHETRONE IN MICE, 6 HR. AFTER ADMINISTRATION OF THE DRUG, ALONE AND WITH PAS

(The results are the means ± S.E. of determinations on groups each of six mice)

Method of Administration	Concentration of Sulphetrone (mg./100 g.) 6 Hr. after Administration of the Drugs											
	Lung	Liver	Kidney	Spleen	Heart	Brain	G.I. Tract	Muscle	Bone	Skin		Blood
										Inj. Site	Remote	
After Oral Administration 0·625 g./kg.												
Alone ..	3·6±0·2	13·0±1·64	7·0±1·64	8·3±3·12	2·6±0·25	1·8±0·38	253± 8·87	1·8±0·3	2·6±0·4	4·9±0·88		1·4±0·11
With PAS	1·9±0·25	4·2±0·17	2·2±0·19	3·2±2·13	1·2±0·24	0·9±0·22	109± 8·52	0·8±0·04	1·1±0·19	1·2±0·13		2·0±0·16
After Intraperitoneal Injection 0·25 g./kg.												
Alone ..	2·4±0·69	20·3±8·6	5·8±1·38	8·1±3·05	1·8±0·93	0·6±0·36	104±21·3	1·9±0·82	2·6±1·7	9·3±0·53	2·4±0·35	0·5±0·26
With PAS	1·5±0·24	4·7±1·02	4·8±1·55	4·7±2·7	0·4±0·26	1·3±1·04	51·8±16·3	3·2±1·98	1·2±0·32	10·7±0·36	1·6±0·6	0·5±0·19
After Subcutaneous Injection 0·25 g./kg.												
Alone ..	3·1±1·15	11·8±2·46	6·8±1·8	6·4±2·91	1·3±0·66	0·8±0·43	79±16·3	1·7±1·07	1·9±0·61	3·4±0·4	2·4±0·49	0·7±0·23
With PAS	2·3±0·63	4·8±1·09	3·9±0·96	2·7±0·84	1·4±0·42	1·6±0·59	41·1± 9·2	1·9±0·85	1·4±0·93	3·2±0·93	2·9±0·98	0·9±0·13

the drug is given either orally or parenterally with PAS are due to the prevention of storage of sulphetrone in the tissues, chiefly in the liver, because of competitive combination of PAS with the liver protein. The fact that the effect is most marked during the first 2 to 3 hr. after administration, when the PAS concentration is at its highest, and diminishes as the PAS is eliminated, supports this. The findings of Way *et al.*, who showed that high concentrations of PAS are attained in the liver 15 min. after intravenous administration to rats, with subsequent fairly rapid fall in concentration, practically no PAS being detected at four hours, adds confirmatory evidence.

In addition some increase in absorption of sulphetrone occurs after oral administration, resulting in a still greater increase in the blood concentration of sulphetrone than occurs after parenteral administration. This, again, may be because PAS prevents the formation of sulphetrone-protein complexes within the intestine, thus making more drug available for absorption.

The Influence of PAS on the Side Effects Produced by Sulphetrone

Although sulphetrone produces no acute toxic effects in man or experimental animals when given by mouth or parenterally, side effects have been observed when the sulphetrone concentration in the blood rises above certain values.

Administration of a single large dose (1 g./kg.) of sulphetrone either by mouth or by intraperitoneal injection to dogs and rabbits generally causes a rise in the alkali reserve as measured by the plasma CO_2-combining capacity, although tests made on rabbits given sulphetrone for long periods showed the animals to be capable of establishing a plasma-alkali balance. A similar equilibrium is established in man (Brownlee, 1948) and no significant change in the CO_2-capacity of the plasma is observed after protracted treatment with sulphetrone.

After a few weeks of treatment, the blood of man and animals containing therapeutic concentrations of sulphetrone is usually dark brown. The pigment has been identified as methaemoglobin. Calculation from the O_2-carrying capacity of the blood shows that the amount of methaemoglobin represents about 4–12% of the total haemoglobin.

It seemed of interest to see if PAS had any influence on these effects, especially as the sulphetrone blood concentrations attained when the two drugs are given together are higher than those given by sulphetrone alone.

Plasma CO_2-combining Capacity

Three dogs were each given a single oral dose of sulphetrone (0.75 g./kg.), a second group of three animals the same dose of PAS and a third group a mixture of the two drugs (0.75 g./kg. of each). At intervals, blood was withdrawn from the saphenous vein into a heparinized syringe, immediately centrifuged under paraffin, and the CO_2-combining capacity determined by the manometric method of Peters and Van Slyke (1932).

The results are given in Table III. An increasing trend in the alkali reserve was shown in all three

TABLE III

EFFECT OF SULPHETRONE AND PAS ON PLASMA CO_2-COMBINING CAPACITY IN DOGS

(The results are the means of determinations on three dogs in each expt. Dose of each drug, 0.75 g./kg. a = sulphetrone concn. (mg./100 ml.) in blood, b = PAS concn. (mg./100 ml.) in blood, c = vol. % CO_2 plasma-combining capacity. * PAS given in divided doses at 0 and 3½ hr.)

Drug		Time After Dosage					
		0 hr.	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.
Sulphetrone alone	a	0	2·4	3·0	4·3	3·3	2·1
	c	50·9	54·1	54·6	56·2	57·3	52·1
PAS alone	b	0	45·5	28·9	16·7	11·4	3·1
	c	52·2	47·0	51·2	52·1	52·4	51·7
Sulphetrone +PAS*	a	0	10·8	13·4	15·9	15·4	4·1
	b	0	25·0	27·0	25·7	23·8	3·7
	c	55·5	56·2	55·5	57·1	58·3	59·3

dogs given sulphetrone alone, the plasma CO_2-combining capacity showing some increase at the first hour in two animals and at the second hour in the third. The effect persisted for at least 6 hr., but 24 hr. after dosage the values did not differ significantly from the original figure.

PAS caused a consistent fall in the plasma CO_2-capacity at one hour after treatment; the value had risen again by the second hour and thereafter showed no significant variation throughout the period of test. This fall in alkali reserve caused by PAS was consistently eliminated when the two drugs were given together, but in two out of the three experiments some increase in the CO_2-capacity was shown from the fourth hour. Thus it seems that initially the two drugs act in a compensatory manner, cancelling the opposite effects on the plasma CO_2-combining capacity; but, as the PAS is eliminated, the delayed effect of the sulphetrone becomes apparent.

Measurement of Methaemoglobinaemia

Because of the reported variation in sensitivity of different species to the action of aromatic amines in producing methaemoglobinaemia (Spicer, 1950), preliminary observations were made on guinea-pigs, rabbits, and dogs to determine the most suitable animals for measurement of the effect of PAS on the methaemoglobinaemia induced by sulphetrone. Guinea-pigs were found to be the most susceptible animals, and methaemoglobin could be detected in their blood within two weeks of their being fed on a diet containing sulphetrone resulting in an average daily intake of drug of 200 mg./kg. or more. No methaemoglobin was found in blood samples from rabbits or dogs given a corresponding dose of sulphetrone for the same period.

Three groups of guinea-pigs, each consisting of three animals, were fed on Coward's diet containing respectively 3% sulphetrone, 6% PAS and a mixture of 3% sulphetrone and 6% PAS. The average daily intake of sulphetrone in animals weighing 400–500 g. was 2.0 g. The animals fed on diets containing PAS ate less than the other groups, so, in a second experiment, the concentrations of both drugs in the diets were reduced to half. The diet was supplemented with 50 g. greenstuff daily. The relative percentage of methaemoglobin to total haemoglobin was determined by comparing the extinction coefficient of the sample with the extinction coefficient after conversion of the pigment to 100% methaemoglobin, as described by Vandenbelt, Pfeiffer, Kaiser, and Sibert (1944). Table IV shows that PAS does not prevent the production of methaemoglobin although the degree of methaemoglobinaemia is no greater in the animals given the combined drugs, in spite of the increased sulphetrone blood concentrations. Methaemoglobin disappears from the blood stream within a week of discontinuing the drugs. The group receiving sulphetrone alone showed a normal gain in weight as compared with the controls. The smaller weight increase shown by group B in the first experiment resulted from a decreased food consumption owing to the taste of the PAS; when the concentration of the latter was reduced in the second experiment, the weight gain was very similar in both groups. Thus it seems that, while PAS does not abolish the side effects produced by sulphetrone, it does not aggravate them, even though the concentration of sulphetrone in the blood is increased.

The finding that one drug may influence the absorption and blood concentration of another may prove of some interest in view of the now well accepted concept of combined chemotherapy in the treatment of those infections where bacterial resistance tends to develop. PAS is frequently prescribed with isoniazid or streptomycin or both, and protein binding occurs with therapeutic concentrations of streptomycin to the extent of 30% (Boxer *et al.*, 1949). Thus the results reported here may have a wider applicability both in these specific applications and possibly in others involving the simultaneous administration of two drugs.

TABLE IV

INFLUENCE OF PAS ON METHAEMOGLOBINAEMIA CAUSED BY SULPHETRONE IN GUINEA-PIGS

The results are the means of determinations on groups of three animals. a = % gain in weight, b = drug concn. (mg./100 ml.) in the blood, c = % methaemoglobin.

Drug and % in Diet		Weeks After Commencing Treatment			
		2	3	4	5
Sulphetrone 3% ..	a	117·8	133·7	140·4	144·3
	b	3·2	3·8	3·8	0 *
	c	19·3	24·1	15·7	0
PAS 6%	a	110·0	110·7	113·2	116·1
	b	3·7	5·1	4·7	0 *
	c	0	0	0	0
Sulphetrone 3% + PAS 6%	a	108·5	114·0	114·7	117·0
	b	8·8 S, 6·4 PAS	5·5 S, 4·2 PAS	6·1 S, 6·2 PAS	0 *
	c	16·4	21·5	21·1	0
Sulphetrone 1·5%	a	111·3	113·4	116·3	120·8
	b	2·5	2·8	1·7	2·7
	c	6·5	14·5	7·0	9·9
Sulphetrone 1·5% + PAS 3%	a	111·1	108·8	114·4	122·3
	b	4·7 S, 2·1 PAS	5·1 S, 3·9 PAS	3·9 S, 2·8 PAS	4·7 S, 2·3 PAS
	c	9·4	14·8	7·1	13·0
Controls	a	126·5	133·7	140·7	150·5
	c	0·04	0·14	0·37	0

* Drug discontinued after fourth week of treatment.

Summary

1. Combined oral administration of PAS and sulphetrone to mice, rabbits, and dogs produces a three- to four-fold increase in sulphetrone concentration in the blood compared with that produced by the same dose of sulphetrone alone.

2. The effect is only partly due to increased absorption from the gut, since a similar but smaller effect is produced when the drugs are given parenterally.

3. Both biliary and urinary excretion of sulphetrone is increased when the drug is given together with PAS, the increased excretion reflecting the increase in blood concentrations.

4. Sulphetrone and PAS are bound to plasma proteins to about the same extent, the degree of protein binding *in vitro* being 87.1% and 85.6% respectively. These values are reduced to 74.8% and 81.9% when both drugs are present and the concentration of PAS is double that of sulphetrone.

5. Tissue distribution studies in mice, 6 hr. after dosage, show that the highest concentrations of sulphetrone are in the gastro-intestinal tract and in the liver, with spleen and kidney next. Concentrations of the drug in all the tissues are considerably reduced by simultaneous administration of PAS.

6. The raised sulphetrone blood concentrations obtained when the drug is administered together with PAS appear to result from competition of the two compounds for a limited number of protein receptors, and the consequent prevention of storage of sulphetrone in the tissues, particularly in the liver. Some increased absorption from the gut also occurs after oral dosage.

7. Combined dosage of the two drugs does not prevent the appearance of side effects due to sulphetrone, although the effect on the CO_2-combining capacity of the plasma is delayed and the degree of methaemoglobinaemia is not increased in spite of the increased blood concentration.

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