ACTA CHEMICA SCANDINAVICA 1 (1947) 605—618

The Solubilities of Separate Sulphanilamides and Their Mixtures („Sulpha-Combination")

BERTIL SJÖGREN and BERTIL ÖRTENBLAD*

The Central Laboratories, Astra, Södertälje, Sweden

The sulphanilamides most effective in clinical practice are all liable to cause injuries to the kidneys owing to the fact that these compounds or their acetyl derivatives are excreted as supersaturated solutions from which concrements may be formed in the renal tubules. Provided that the sulphanilamides do not affect each other's solubility but dissolve independently, the risk of concrement formation might be almost eliminated if, for the attainment of a certain blood concentration or effect, not merely one compound but a mixture of several equivalent sulphanilamides were employed. From the clinical standpoint it cannot make any difference if several compounds are used instead of one when all the drugs are equally effective. The risk of concrement formation will, however, be reduced in this case, since each drug in the mixture is excreted in a considerably lower degree of saturation than when a single compound is employed.

This new principle of treatment has been called »sulpha-combination». It was first developed and used in Sweden. At an early date Hagerman started his clinical tests with a combination of two compounds since we had stated that the sulphanilamides do not exert any mutual solubility effects [1]. Later Frisk, Hagerman, Helander and Sjögren published extensive experimental proofs [2]. In these investigations the solubilities of the most suitable sulphanilamides and their acetyl derivatives separately and in mixtures were determined. Also experiments on the concrement formation in animals and determinations of absorption and excretion were carried out. The formula of the most suitable mixture, »sulphadital», was given and its actions and uses described. This mixture contains sulphathiazole, sulphadiazine, and sulpha-

* The authors are indebted to Mr. Yngve Ekhamn for technical assistance.

merazine in certain proportions. Independently of these authors the same idea was developed by Lehr in U. S. A., who has also demonstrated the practicability of the method [3].

In our earlier publications data on the solubility of some important sulphanilamides and their acetyl derivatives, separately and in mixtures, have been given. However, it was not possible to publish all details before. A more complete account may be of twofold interest. The results constitute the main basis of the »sulpha-combination» principle but they are also of interest from a chemical point of view. Since the results of earlier determinations of the solubilities of single sulphanilamides are very divergent and the cause of these differences had to be cleared up. To the compounds earlier investigated, we added elkosin and its acetyl derivative, as they are said to have particularly suitable solubility properties [4, 5].

We have also used our rather extensive data to calculate the acid dissociation constants (pK_a) and the solubilities of the undissociated compounds (S_0). These calculations are based on the simple laws of solubility following the procedure of Krebs and Speakman [6]. The majority of the sulphanilamides are ampholytes. However, within the physiological pH range, the basic functions have been disregarded and the compounds considered as weak monobasic acids. With knowledge of pK_a and S_0 the solubility may be calculated at various pH values. By comparing these values with the experimental ones it is possible to test the validity of this conception.

METHODS

The solubilities of the following compounds have been determined separately and in mixtures: sulphathiazole (2-sulphanilamido-thiazole), sulphadiazine (2-sulphanilamido-pyrimidine), sulphamerazine (4-methyl-2-sulphanilamido-pyrimidine) and their N^4-acetyl derivatives; also the separate solubilities of elkosin (6-sulphanilamido-2,4-dimethyl-pyrimidine) and its N^4-acetyl derivative. Fresh urine (a mixture from 11 men) was used as solvent for all compounds and mixtures of compounds. For the free sulphanilamides and for certain of the acetyl derivatives a phosphate buffer (usually $M/30$) were also used and in some cases diluted sodium hydroxide. The determinations of the solubility were carried out at pH 5.9—7.9 and in a few cases also outside this range. The desired pH value of the urine was obtained by the addition of the necessary amount of hydrochloric acid, sodium carbonate, or sodium hydroxide. A small amount of toluene was added to the urine as a preservative. This does not influence the results. The solubilities are given in mg of dissolved substance per 100 ml of solvent (mg %).

In order to determine the concentration of the dissolved substance, the solutions were suitably diluted, the acetyl derivatives hydrolysed and the compound determined according to the method of Bratton and Marshall [7]. Small or negligible corrections were made for the extinction of the reagents and the solvent. From the values of extinction, the amount of substance per 100 ml solution was found by interpolation of standard curves of known solutions.

This method of calculation is more complicated when dealing with the mixtures. The wave length of the maximum colour intensity is not the same for all three sulphanilamides used in the mixtures. The difference, however, is so slight that the curves almost coincide. Thus it is impossible to distinguish between the sulphanilamides in the mixtures. If sulphadiazine is read as sulphathiazole an error of only — 3 % is made. Similarly using sulphamerazine, the error is + 3 %. Thus the errors of the determination of a mixture of sulphadiazine, sulphathiazole and sulphamerazine mutually compensate each other provided the concentrations of sulphadiazine and sulphamerazine are about the same.

In working with mixtures of the free sulphanilamides and their acetyl derivatives, the free sulphanilamides are determined first and calculated as sulphathiazole (f mg %). Then the acetyl derivatives are hydrolysed and the compounds all together determined as sulphathiazole (s mg %). The difference ($s - f$) is converted into acetyl sulphathiazole (a mg %). The total content of dissolved substance is then given by the equation $t = a + f$.

When determining solubility values, special care must be taken to obtain saturation but to avoid supersaturation of the solution. This can be done by working at constant temperature. Either the solid substance may be dissolved in the solvent or a solution may be supersaturated and allowed to become stabilized. In most cases we used an excess of the finely powdered substance.

The solutions were made in Erlenmeyer flasks with glass stoppers, shaken mechanically and kept at 37° C in a water bath for at least 24 hours. Samples were filtered and analysed for concentration and pH.

In the case of sulphathiazole, elkosin and their acetyl derivatives the solubility was also determined after the crystallisation from a supersaturated solution in 1/30 M phosphate buffer. These solutions were made by saturation at 100° C followed by mechanical shaking at 37° C until the excess of substance had crystallized out. At different times samples of the clear solution were analysed for concentration and pH. — Some determinations of concentrations were also made of supersaturated solutions of acetyl sulphathiazole and acetyl elkosin. In these cases the solutions were cooled down in 2 min. to 37—38° C, then shaken at 37° C in the same manner as mentioned above. Analyses were made immediately after cooling down, and also after 15 and 30 minutes. This was done in order to compare the ability of the compounds to form supersaturated solutions.

The pH determinations were made with a glass electrode using a standard buffer solution. To simplify the procedure, an aliquot was withdrawn for the determination of the concentrations and the solutions were then allowed to cool to 20° C before the pH was measured. No precipitate was formed during this short period of cooling. The pH did not vary appreciably between 37 and 20° C. With the pH-meter used the error did not exceed ± 0.03 units.

The measurements of extinction values have been carried out with a single cell photoelectric colorimeter according to Brunius*. The errors in these measurements, the errors

* Constructed in the State Institute for Public Health, Stockholm, Sweden.

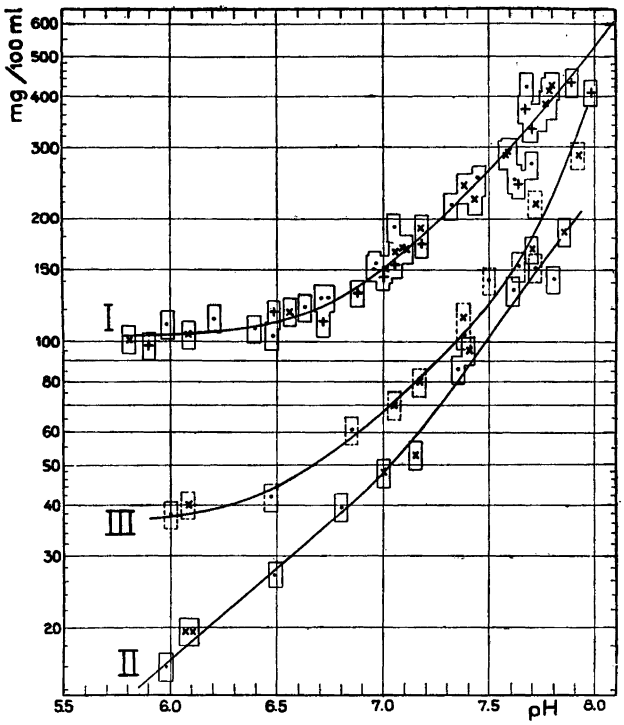

Fig. 1. The solubility at 37° C of:
I Sulphathiazole.
II Sulphadiazine.
III Sulphamerazine.
Solvents: Phosphate buffer (x), urine (·) and diluted sodium hydroxide (+).

in the dilutions necessary for the determination, and other possible errors will give a rather great total error. Quadruple determinations of sulphathiazole at pH 6.10 were, for example, 107, 100, 107 and 101 mg %. It may be concluded that in the case of a single free sulphanilamide, the possible maximal error can amount to ± 8 %, while in a mixture of three substances it amounts to ± 10 %. The same values for the acetyl derivatives would be ± 10 % and ± 12 % respectively. The last figure is also applicable to mixtures of all six compounds.

No consideration was given to the accuracy of the pH measurements when calculating the above errors. If only two determinations are made, a difference of 0.06 units cannot be distinguished with any certainty with the pH-meter used. At a pH of 6 or less this is of negligible importance. With an increasing pH the slope of the solubility curves grows steeper and a difference of 0.06 units may be of great influence on the results. For example, a change of the pH from 7.94 to 8.00 results in an increase of the solubility of acetyl sulphathiazole from 78 to 98 mg %, the difference being about 20 %.

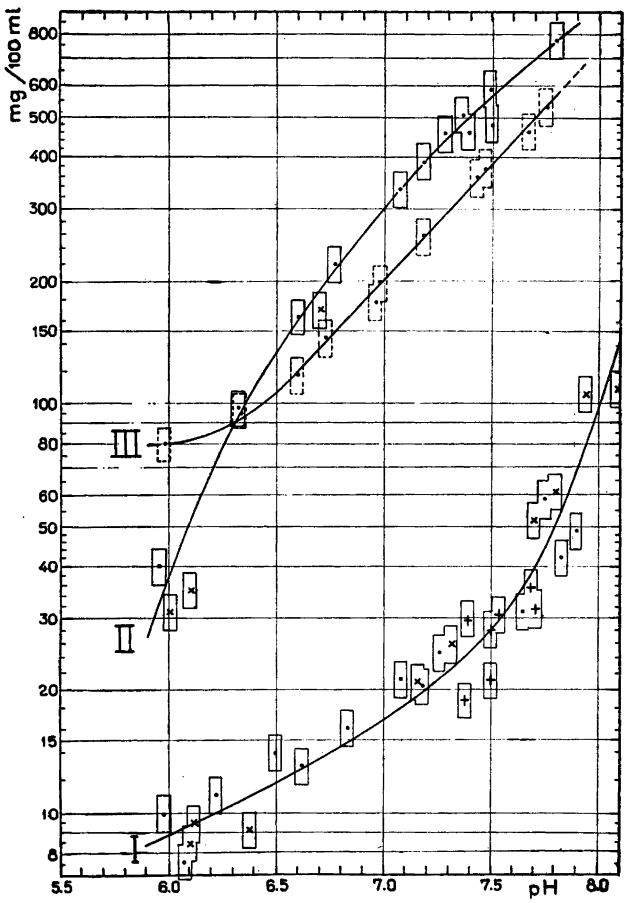

Fig. 2. Solubility at 37° C of:
I Acetylsulphathiazole.
II Acetylsulphadiazine.
III Acetylsulphamerazine.
Solvents: Phosphate buffer (x), urine (·) and diluted sodium hydroxide (+).

RESULTS

The experimental values of the solubility are plotted against the pH on a logarithmic scale. Each point is surrounded by a rectangle graphically showing the maximal error of the determination. The values for each substance are plotted in the same system, this being possible due to the fact that the solubilities at a certain value of pH seem to be independent of the three solvents used. The different solvents are indicated by different signs. From

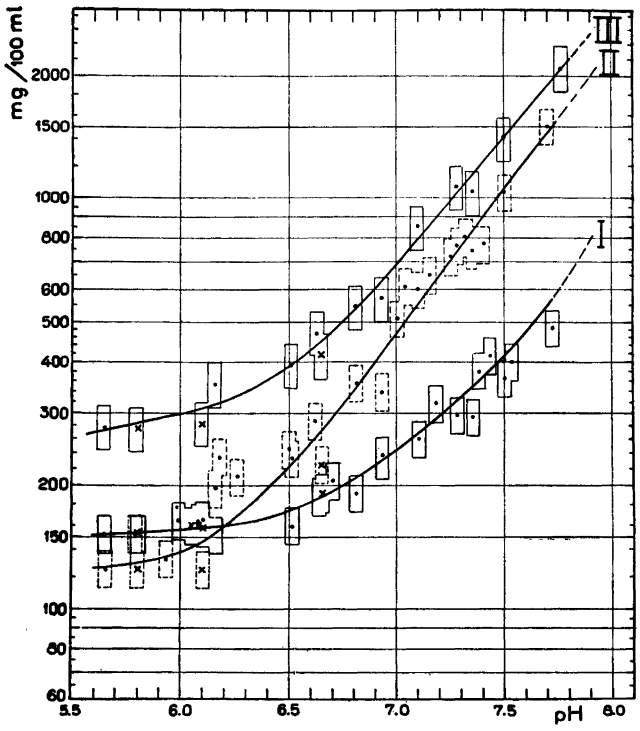

Fig. 3. The solubility at 37° C of the sulphanilamides and their acetyl derivatives in mixtures:
I Sulphathiazole + Sulphadiazine + Sulphamerazine.
II Acetylsulphathiazole + Acetyl sulphadiazine + Acetyl sulphamerazine.
III Sulphathiazole + Sulphadiazine + Sulphamerazine and their acetyl derivatives.
Solvents: Phosphate buffer (x) and urine (·).

the curves the solubility at various pH values can be read. The solubilities so obtained are to be considered as averages.

Fig. 1 shows the solubilities of sulphathiazole, sulphadiazine and sulphamerazine at pH 5.9—7.9 and 37° C, Fig. 2 the corresponding values for the acetyl derivatives. The curves illustrate, as is already known, that sulphathiazole is slightly more soluble than the two sulpha-pyrimidine compounds. They also show that the solubility increases strongly with increase in pH. Sulphathiazole is more soluble than its acetyl derivative. In the case of sulphadiazine and sulphamerazine the opposite is true.

The most important question is, however, to ascertain the extent to which the compounds influence each other's solubility. Fig. 3, Tables 1 a and 1 b show the results of these determinations at pH 5.5—7.9. It is quite evident

Table 1 a. The solubility, in mg/100 ml at 37° C, of sulphathiazole, sulphadiazine, sulphamerazine, and their acetyl derivatives, separately and in mixtures.

Substances	Source of values		Solubility at pH					
			5.5	6.0	6.5	7.0	7.5	7.9
Sulphathiazole	Fig. 1		103	105	111	151	263	450
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	105	110	120	150	250	450
		pK_a and S_0 according to Krebs and Speakman	95	100	115	170	320	690
Acetyl sulphathiazole	Fig. 2		7.1	8.8	11.5	17	28	69
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	7.5	8.0	10.3	17	37	85
		pK_a and S_0 according to Krebs and Speakman	7.5	8.2	10.5	18	41	95
Sulphadiazine	Fig. 1		10	17	28	48	104	200
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	13	15	23	49	130	320
		pK_a and S_0 according to Krebs and Speakman	12	15	27	63	185	440
Acetyl sulphadiazine	Fig. 2		10	3	34	305	565	850
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	20	37	95	290	900	—
		pK_a and S_0 according to Krebs and Speakman	27	45	100	285	850	—
Sulphamerazine	Fig. 1		37	38	44	68	127	310
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	36	38	45	65	135	290
		pK_a and S_0 according to Krebs and Speakman	43	46	56	88	190	410
Acetyl sulphamerazine	Fig. 2		79	80	105	202	388	650
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	75	82	105	180	400	900
		pK_a and S_0 according to Krebs and Speakman	89	105	140	310	800	1950

Table 1 a (continued)

(Sulphathiazole +sulphadiazine +sulphamerazine)	Fig. 3	153	158	173	245	420	800
	Fig. 1	150	160	183	267	494	960
Acetyl- (sulphathiazole + sulphadiazine + + sulphamerazine)	Fig. 3	126	138	222	470	1070	2000
	Fig. 2	96	127	251	524	981	1569
(Sulphanilamides + acetyl derivatives)	Fig. 3	265	300	390	695	1420	2550
	Figs. 1 and 2	246	286	434	791	1475	2529
	Fig. 3, curves I and II	279	296	395	715	1490	2800

that the three compounds of sulphadital dissolve independently of one another. This is the case, at least within this pH range, regarding a mixture of the three sulfa drugs, a mixture of their acetyl derivatives, and a mixture of all six compounds. The calculated and experimental values are in good agreement (Table 1 b). The results agree with the theoretical expectations, because the substances even in saturated solutions are present in very low concentrations and a decrease of solubility by means of influencing the activity coefficients is hardly possible. With the pH constant and only small changes of the concentration of neutral salts the magnitude of the total concentration of the common ions is always the same.

When making a good mixture for clinical use, the choice of constituents is of primary importance. From various points of view, the mixture used in our experiments has been found to be the most suitable one. Another important point is the proportions of the components. Besides the solubility of the free sulphanilamides and their acetyl derivatives, many pharmacological questions have to be considered. These have been discussed in a previous paper [2]. The most ideal composition hitherto has been found to be: sulphathiazole 37 %, sulphadiazine 37 % and sulphamerazine 26 %. With normal doses of this mixture none of the excreted products in the urine exceed their solubility limits, if the reaction is approximately neutral [2].

Our investigations on elkosin show that this compound has a high solubility while its acetyl derivative has a low one, the solubility of the latter being about the same as that of acetyl sulphathiazole (Fig. 4, Table 2). Meier, Allemann and v. Meyenburg have found much higher solubilities for these compounds [4]. They determined the solubility of the acetyl derivative in the usual ways *i. e.*, by dissolving the substance or by crystallizing the substance

Table 1 b. Ratio between found and calculated values for the solubility of mixtures over the pH-range 5.5—7.9. The quotients in parenteses include extrapolated values.

Substances	The quotient calculated	pH					
		5.5	6.0	6.5	7.0	7.5	7.9
Sulphathiazole + sulphadiazine + sulphamerazine	Solubility according to Fig. 3 / Solubility calculated from Fig. 1	(1.02)	0.99	0.95	0.92	0.85	(0.83)
Acetyl sulphathiazole + acetyl sulphadiazine + acetyl sulphamerazine	Solubility according to Fig. 3 / Solubility calculated from Fig. 2	(1.31)	1.09	0.88	0.90	1.09	(1.27)
Sulphathiazole + sulphadiazine + sulphamerazine + their acetyl derivatives	Solubility according to Fig. 3 / Solubility calculated from Fig. 1 and 2	(1.08)	1.05	0.90	0.88	0.96	(1.01)
	Solubility according to Fig. 3 / Solubility calculated from Fig. 3, curves I and II	(0.95)	1.01	0.99	0.97	0.95	(0.91)

from a supersaturated solution. In the latter case very high values were obtained (Table 2). Evidently these authors believe that the acetyl derivative of elkosin has an especially high ability to remain in a supersaturated solution. From a practical point of view this would be important. However, we could not verify these findings. As is seen from Tables 3 and 4 this property is not more pronounced than in the case of acetyl sulphathiazole.

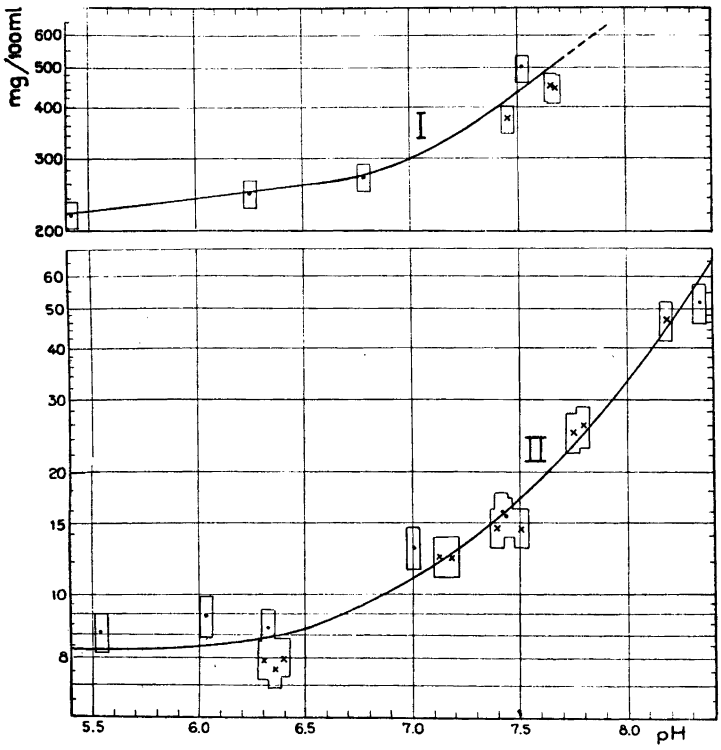

Fig. 4. The solubility at 37° C of:
I Elkosin.
II Acetyl elkosin.
Solvents: Phosphate buffer (x) and urine (·).

Table 2. The solubilities, in mg/100 ml at 37° C, of elkosin and acetyl elkosin.

Substance	Source of values		Solubility at pH					
			5.5	6.0	6.5	7.0	7.5	7.9
Elkosin	Fig. 4		222	238	256	300	435	620
	Krebs' and Speakman's nomogram and our pK_a and S_0		220	225	245	290	440	800
	Meier, Alleman, and v. Meyenburg		362	—	600	—	1100	—
Acetyl elkosin	Fig. 4		7.3	7.5	8.4	11.0	17.5	29
	Krebs' and Speakman's nomogram and our pK_a and S_0		7.5	7.8	8.5	11.0	18.5	35
	Meier, Alleman, and v. Meyenburg	from supersaturated solution	190	—	224	—	485	—
		by direct dissolving substance	24	—	24	—	39	—

Table 3. The solubility, in mg/100 ml at 37° C, of sulphathiazole, elkosin, and their acetyl derivatives determined by crystallizing the substance from supersaturated solutions or by direct dissolving the substance.

Substance	Method of preparing saturated solutions	Time for stabilizing of solutions, days	pH at 20° C	Solubility	
				Found	Calculated from solubility curve
Sulphathiazole	From supersaturated solution	1	7.12	186	170
	» » »	2	7.11	169	170
	» » »	1	7.48	280	258
	» » »	2	7.45	250	248
	» » »	1	7.73	360	355
	» » »	2	7.74	352	360
	By direct dissolving substance	1	7.78	380	380
	» » » »	2	7.78	380	380
	From supersaturated solution	1	7.89	436	445
	» » »	2	7.89	428	445
	By direct dissolving substance	1	8.08	588	600
	» » » »	2	8.07	616	590
Acetyl sulphathiazole	From supersaturated solution	1	7.50	27	28
	» » »	2	7.48	28	27
	By direct dissolving substance	1	7.55	35	30
	» » » »	2	7.59	36	32
Elkosin	From supersaturated solution	1	7.45	402	420
	» » »	2	7.45	370	420
Acetyl elkosin	From supersaturated solution	1	6.29	7.5	7.8
	» » »	2	6.35	6.9	8.0
	By direct dissolving substance	1	6.39	7.0	8.1
	From supersaturated solution	1	7.16	14.5	12.5
	» » »	2	7.12	12.3	12.2
	By direct dissolving substance	1	7.00	13.0	11.0
	From supersaturated solution	1	7.71	28	22
	» » »	2	7.74	25	23

According to Gsell, the concentration of elkosin in the urine after a dose of 3 grams daily is 100—300 mg % of the free compound and 150—400 mg % of the total compound (*i. e.* free plus acetylated compound)[5]. After 5 grams daily, the corresponding concentrations are 300—500 mg %, and 400—700 mg % respectively. The average degree of acetylation in the urine is said to be about 20 % (12—35 %). If these concentrations are compared with the solubilities at various pH values found by us, it is evident that after a dose of 3 grams daily free elkosin does not necessarily appear in a supersaturated state.

Table 4. Concentrations of acetyl sulphathiazole and acetyl elkosin in supersaturated solutions. Parallel determinations have same numbers.

Determination	Substance	Supersaturated solutions kept at 37°, minutes	pH	Concentration of substance		Solubility from curve
				mg/100 ml	% of zero value	
1	Acetyl sulphathiazole	0	6.28	26	100	10.8
	»	15	6.28	18	69	10.8
	»	30	6.28	14	54	10.8
2	»	0	6.78	111	100	14.3
	»	15	6.78	63	57	14.3
	»	30	6.78	48	44	14.3
3	»	0	7.58	116	100	32
	»	15	7.62	102	88	34
	»	30	7.64	82	71	35
1	Acetyl elkosin	0	6.28	39	100	7.8
	»	15	6.29	27	69	7.8
	»	30	6.30	21	54	7.9
2	»	0	6.83	67	100	9.9
	»	15	6.87	52	78	10.1
	»	30	6.82	43	64	9.8
3	»	0	7.68	204	100	21.5
	»	15	7.70	137	67	22.0
	»	30	7.74	106	57	23.0

After a dose of 5 grams daily, the concentrations of free elkosin in the urine is always higher than its solubility up to a pH value of about 7. Acetyl elkosin, however, is always excreted as a strongly supersaturated solution. If 20 % of the lowest dose and the lowest total concentration (150 mg %) were acetylated, then the acetyl derivative would appear in a concentration of 30 mg %. Even this value is about 3 times as high as the solubility at pH 7. At a lower pH value, the difference is still greater. As the acetyl derivative has such a low solubility, it is probable that elkosin will give the same renal complications as other single sulphanilamides.

From the solubility curves for the single compounds (Figs 1 and 2) we have obtained the solubilities at various pH values between 5.9 and 7.9 in order to calculate pK_a and S_0 according to Krebs and Speakman. It is assumed that the compounds appear as sparingly soluble mono-basic acids. If the solu-

Table 5. Average pK_a and S_0 values. The standard errors are obtained in the usual manner $\left(E = \sqrt{\frac{\Sigma \Delta^2}{n\ (n-1)}}\right)$.

Substance	pK_a according to		S_0 according to	
	our determinations	Krebs and Speakman	our determinations	Krebs and Speakman
Sulphathiazole	7.37 ± 0.03	7.10	103 ± 2	92
Acetyl sulphathiazole	6.87 ± 0.06	6.81	7.1 ± 0.4	7.1
Sulphadiazine	6.47 ± 0.07	6.28	11 ± 1	9.9
Acetyl sulphadiazine	5.6 ± 0.3	5.86	10.5 ± 1	18.6
Sulphamerazine	7.05 ± 0.03	6.95	35 ± 0.5	41
Acetyl sulphamerazine	6.84 ± 0.04	6.55	71 ± 3	80
Elkosin	7.48 ± 0.04	—	218 ± 6	—
Acetyl elkosin	7.30 ± 0.03	—	7.0 ± 0.2	—

bility at pH 5.9 is S' and the other ten solubilities in turn are S'', K_a values for each substance may be calculated from the equation:

$$K_a = \frac{S'' - S'}{\frac{S'}{[H]''} - \frac{S''}{[H]'}}$$

The single values showed a certain trend indicating that the compounds did not exactly follow the theoretical solubility curve for mono-basic acids. Especially in the case of acetyl sulphadiazine was this pronounced. From the average value of pK_a and the solubilities from our curves *(S)* at various pH a series of S_a values were calculated according to the following equation:

$$S_0 = \frac{S}{1 + 10^{pH-pKa}}$$

The average pK_a and S_0 values are given in Table 5. The solubilities calculated in this way agree rather well with those obtained from the experiments reported in Tables 1 and 2. From this it may be concluded that, for practical purposes, it is possible to consider the sulphanilamides as mono-basic acids, although this may not be exactly true from a chemical point of view. We

thus are able to confirm the opinion of Krebs and Speakman that knowing the values of pK_a and S_0 the solubilities may be calculated with satisfactory accuracy within the physiological range of pH.

SUMMARY

The solubilities of sulphathiazole, sulphadiazine, sulphamerazine and the corresponding N^4-acetyl derivatives were determined at the pH-range 5.9—7.9, in different solvents saturated with the compounds separately and with their mixtures. The compounds do not affect each other's solubility. This is the main basis for the »sulpha-combination» principle.

The solubilities of elkosin and N^4-acetylelkosin were also determined and discussed.

The acid dissociation constants (pK_a) and the solubility of the undissociated compounds (S_0) were calculated.

REFERENCES

1. Hagerman, G. *Nord. Med.* **22** (1944) 1223; **24** (1944) 1944.
2. Frisk, A. R., Hagerman, G., Helander, S., and Sjögren, B. *Nord. Med.* **29** (1946) 639; *Brit. Med. J.* (1947) 7.
3. Lehr, D. *Proc. Soc. Exp. Biol. and Med.* **58** (1945) 11; **64** (1947) 393; *J. Urology* **55** (1946) 548.
4. Meier, R., Allemann, O., and v. Meyenburg, H. *Schweiz. med. Woch.* **74** (1944) 1091.
5. Gsell, O. *Schweiz. med. Woch.* **74** (1944) 1095.
6. Krebs, H. A., and Speakman, J. C. *Brit. Med. J.* (1946) 47.
7. Bratton, A. C., and Marshall, E. K. *J. Biol. Chem.* **128** (1939) 537.

Received July 20, 1947.