a pure state, and which will produce a consistent colour with the Folin & Ciocalteu reagent. Phenol is such a substance and it has been used to provide the colour standard in this work. Thus the preparation of a pure sample of tyrosine is avoided.

Since the relationship between concentration of phenol and the colour produced in the reaction with the phenol reagent is not linear over a wide range, peptic activity has been related to a standard phenol at one point only. A small divergence from true proportionality is also found for the mixture of tyrosine and tryptophan estimated in the digestion products. Herbert (1946) has confirmed this point for the estimation of phenol with Folin & Ciocalteu's phenol reagent when $NaCO_3$ is used as the alkaline medium for colour development.

The method has proved quite practicable with ordinary citrated plasma so that, in the absence of a supply of dried plasma or serum, it could be used with citrated or whipped ox blood. However, it is unlikely that the supply of plasma or serum will fail, since occasional batches will always be rejected as unfit for transfusion because they are icterogenic. A new relationship between peptic activity and colour would, however, have to be established for fresh plasma. Any alteration of the concentration of the substrate beyond the range 5–6 g./100 ml. would also require a simultaneous modification of the strength of the trichloroacetic acid to ensure that the optimal pH was reached in the colour development reaction.

The method described estimates the total proteolytic activity of gastric juice at pH 2·1 with one special substrate. The practice is to attribute all this activity to pepsin, and the method has therefore been described as a method for measuring pepsin. However, the work of Northrop (1939) suggests that it may later prove necessary to apportion the activity of gastric juice to pepsin, gelatinase and possibly other enzymes. The uncertainty surrounding the source of the activity of the gastric juice seemed in part to justify the completely arbitrary measure of proteolytic activity used in this work.

SUMMARY

1. A method is described for measuring the proteolytic activity of gastric juice with a substrate of dried plasma or serum protein which has been found to be reproducible and convenient.

2. The method allows of the comparison of peptic activity in different laboratories without reference to a standard pepsin.

REFERENCES

Anson, M. L. (1938). *J. gen. Physiol.* **22**, 79.
Anson, M. L. & Mirsky, A. E. (1932). *J. gen. Physiol.* **16**, 59.
Beazell, J. M., Schmidt, C. R., Ivy, A. C. & Monoghan, J. F. (1938). *Amer. J. digest. Dis.* **5**, 661.
Bucher, G. L., Grossman, M. I. & Ivy, A. C. (1945). *Gastroenterol.* **5**, 501.
Folin, D. & Ciocalteu, V. (1927). *J. biol. Chem.* **73**, 627.
Herbert, F. K. (1946). *Quart. J. Med.* **15**, 221.
King, E. J. (1946). *Microanalysis in Medical Biochemistry.* London: Churchill.
Northrop, J. H. (1939). *Crystalline Enzymes.* New York: Columbia University Press.

The Persistence in the Blood Stream of some Compounds Related to Suramin

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Suramin (Antrypol, Germanin, Bayer 205, Moranyl, F. 309, Belganyl, Naganol, see formula I) displays an extraordinarily marked persistence in the blood stream of man and experimental animals. This property was recognized soon after suramin was first discovered, first, from the long duration of its prophylactic action (Mayer & Zeiss, 1920; Levaditi & Klarenbeek, 1927); secondly, from measurements of the trypanocidal activity of serum and urine of treated animals (Mayer, 1922; Mayer & Menk, 1922). The development of adequate chemical methods of determining it (Steppuhn & Utkin-Ljubovzov, 1924; Lang, 1931; Dangerfield, Gaunt & Wormall, 1938*a*; Boursnell, Dangerfield & Wormall, 1939; Vierthaler & Boselli, 1939), and their use in studying the persistence quantitatively (Lang, 1931; Dangerfield, Gaunt & Wormall, 1938*b*; Boursnell, *et al.* 1939; Vierthaler & Boselli, 1939; Hawking, 1940*a*, *b*) showed that in man, dogs and rabbits, it may persist in the blood stream for as long as 3 months. The

form of the blood concentration-time curve is asymptotic (Lang, 1931); during the first few hours after intravenous administration the concentration falls rapidly, but subsequently more and more slowly, until it may be regarded as almost constant over periods of a few days.

Some work has been done on the biochemical and physicochemical characteristics of suramin responsible for this remarkable persistence. No depot of suramin appears to be formed in any tissue. In general, the tissue concentrations are of the same order as, or lower than, the plasma concentration (Zeiss & Utkina-Ljubovtzova, 1930; Boursnell & Wormall, 1939). It must therefore be assumed that the kidney is able to excrete suramin only very slowly, although it can be detected in the urine during the first few days after dosing (Boursnell *et al.* 1939). The most widely held theory advanced to account for this slow excretion is that suramin is bound to protein, either in plasma or cells. Mandel & Steudel (1926) suggested that it was combined in the body with basic proteins of the histone type. They demonstrated that such combination could occur *in vitro*. Collier (1926) showed that suramin in 1% (w/v) concentration could protect horse serum from heat coagulation, and that it combined with globulins (Collier, 1926, 1927). Brunelli (1934) showed that it combined with the euglobulin fraction of horse serum and, to a less extent, with the pseudoglobulin fraction. Boursnell & Wormall (1939) considered that proteins other than the globulins might also be involved; and Dewey & Wormall (1946) demonstrated the combination *in vitro* of suramin with proteins of various types. They precipitated the protein with alcohol in the presence of suramin, and then extracted the protein-suramin complex with ethanol in a Soxhlet apparatus. Large amounts of suramin remained bound to the protein, even after this treatment. It seems likely that the persistence is largely due to this firm combination with the body proteins; however, other factors may be involved. Many drugs that are largely bound to protein are rapidly eliminated, their slow filtration through the glomeruli being augmented by participation of the tubular epithelium in the renal excretory processes. Examples are sulphathiazole, *p*-aminobenzoic acid (Lundquist, 1945) and penicillin (Beyer, Peters, Woodward & Verwey, 1944; Rantz, Kirby & Randall, 1944; Beyer, 1947). Conversely, the most persistent sulphonamides are those that are extensively reabsorbed from the glomerular filtrate by the tubular epithelium (Fisher, Troast, Waterhouse & Shannon, 1943; Earle, 1944). Usually they are also bound to plasma protein. Tubular processes may possibly play a similar part in the persistence of suramin. The strength of the bond uniting it to protein may also be of importance (Gregerson & Rawson, 1943; Rawson, 1943). A further possibility, which does not seem to have received attention, is that suramin exists in solution as colloidal aggregates of sufficiently high nominal molecular weight to hinder passage through the glomerulus. Some support is provided by the known inability of suramin to pass through a collodion membrane (Boursnell *et al.* 1939).

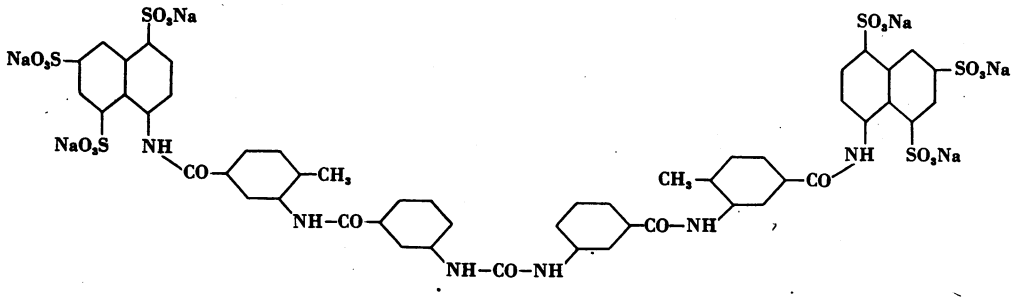
The high persistence of suramin raises interesting questions regarding its degradation by the body. No drug so far adequately examined has proved to be completely unaffected by metabolic processes. If suramin is degraded it must be assumed, either that the process involved is extremely slow, or that the products are themselves highly persistent. The prophylactic action of suramin is associated with the maintenance of a definite blood concentration of about 1·3 mg./100 ml., as determined by the Wormall method (Vierthaler & Boselli, 1939). It is possible that part of this concentration may be contributed by degradation products. The most obvious method of degradation of suramin, a polyamide related in structure to the natural polypeptides, is by hydrolysis to the three constituent aromatic amino-acid residues. The possibility that these are formed *in vivo* was considered by Dewey & Wormall (1946) who showed that a mixture of them, prepared by acid hydrolysis of suramin, was rapidly eliminated. If the postulated degradation gives the constituent amino-acids, it must proceed very slowly. The further possibility that degradation might stop at an intervening stage, yielding a persistent urea or amide, was not examined.

It seemed to the author that an examination of the persistence of compounds structurally related to suramin might provide information on three main problems of interest. First, it might indicate the structural features of the suramin molecule that confer high persistence. Such information would be of general value in the synthesis of prophylactic drugs. Secondly, it might show whether the structural features conferring high persistence are also those conferring high activity. If this were so it could be tentatively assumed that the activity of suramin involves union with an enzyme of the parasite, qualitatively similar to its union with the plasma proteins. Thirdly, if potential hydrolysis products of suramin were examined, their persistence would indicate whether they were likely to be formed *in vivo*.

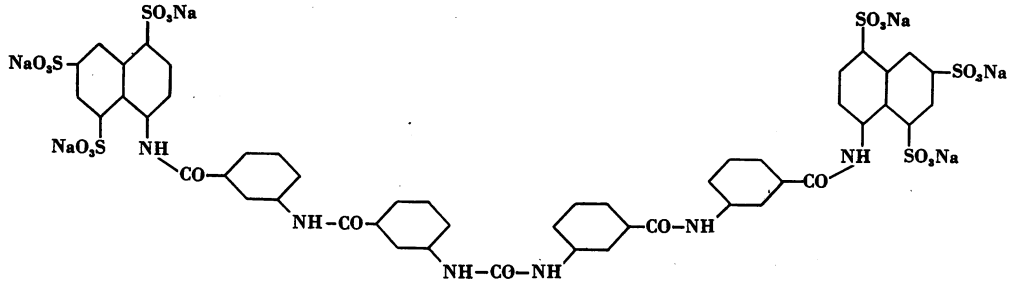
EXPERIMENTAL

Twelve compounds in all were examined. Their structural formulae are shown below. Since they are highly complex, they are referred to throughout by code numbers. Most of them were prepared in these laboratories by Dr R. A. Wilkinson, to whom the author is very grateful.

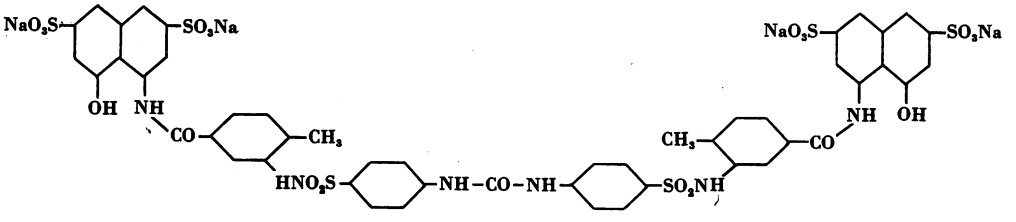
Three methods were used for determining the compounds, based on the standard techniques of diazotization and coupling used in these laboratories be devised. When hydrolysis was necessary, alkaline conditions were used, because the final colour given by normal plasma was thereby reduced. Since some



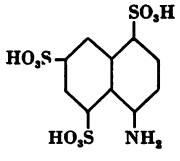
I (suramin)



II (demethylated suramin)

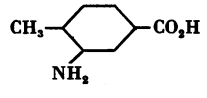


III

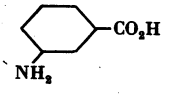


(as a mixed disodium salt)

IV



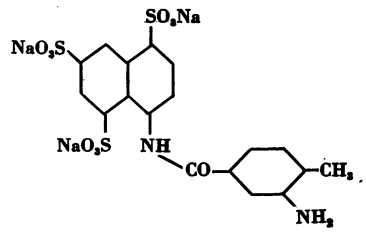
V



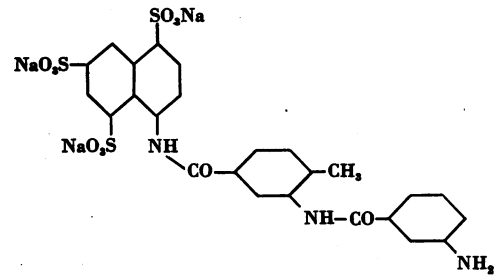
VI

(Rose & Bevan, 1944; Rose & Spinks, 1946; Spinks & Tottey, 1946). They represent the first sets of conditions tried that gave adequate (±10%) recoveries, and are probably not the best that could of the compounds were rapidly eliminated, this advantage outweighed those of acid hydrolysis described by Dangerfield *et al.* (1938*a*). It should be particularly noted that no attempt was made to

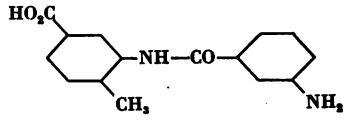
discover the shortest possible time of hydrolysis; 6 hr. was regarded as ample time and used in all experiments.



VII



VIII



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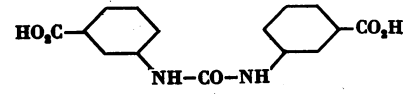
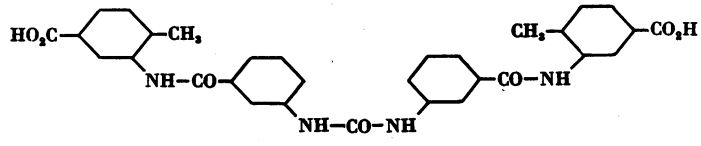
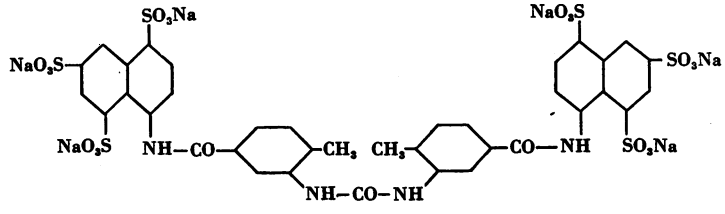


X



XI



XII

Method A, used for compounds V, VI, VII, VIII and IX

Plasma (0·2 ml.) was pipetted into 2 ml. of water in a tube graduated at 10 ml., and treated with 2 ml. of 15% (w/v) trichloroacetic acid. The tube was shaken, allowed to stand for 5 min. and centrifuged at 2000 r.p.m. for 10 min., 0·5 ml. of 0·1% (w/v) $NaNO_2$ was then added; the solutions were well mixed, and adhering protein was removed from the walls of the tube with a thin rod. The tube was then recentrifuged for 5 min. Exactly 20 min. after adding nitrite, 0·5 ml. of 1% (w/v) *N*-β-sulphatoethyl-*m*-toluidine and 2 ml. of 30% (w/v) sodium acetate.$3H_2O$ were added, and the solution was well mixed without disturbing the protein clot. After 20 min. 0·5 ml. of conc. HCl was added, and the volume was made up to the mark with distilled water. The solution was again stirred and the tube was recentrifuged for 5 min. The colour was then read in a photoelectric colorimeter against a series of standards similarly prepared from 0·2 ml. volumes of aqueous solutions containing 0–50 mg. of the appropriate compound/100 ml.

Notes. Coupling of each diazonium compound was approximately complete after 20 min. under the conditions described. A 1 cm. cell and a blue-green filter were used in the colorimeter.

Method B, used for compound IV

Method B was identical with method A except that coupling was allowed to proceed for 40 min. after adding acetate. Even then it was less than 50% complete. The

slow coupling of diazotized 1-naphthylamine-4:6:8-trisulphonic acid (IV) is probably due to steric hindrance by the bulky group in the 8-position. The colours produced were very weak. A 2 cm. cell and a green filter were used in the colorimeter.

Method C, used for compounds I, II, III, X, XI and XII

Plasma (1 ml.) was treated with 2 ml. of 2N-NaOH in a tube graduated at 10 ml.; the tube was stoppered with a vaselined glass stopper, and heated at 100° for 6 hr. After cooling, 1 ml. of conc. HCl was added, and the volume was made up to 10 ml. with distilled water; 2 g. of kaolin (B.P.) were then added, and the tube was well shaken for 1 min. The contents were filtered, and 2 ml. of the almost colourless filtrate were treated with 0·5 ml. of 0·1% (w/v) $NaNO_2$ in a tube graduated at 10 ml. After 20 min. 0·5 ml. of 1% (w/v) *N*-β-sulphatoethyl-*m*-toluidine and 1 ml. of 30% (w/v) sodium acetate ($3H_2O$) were added. Coupling was allowed to proceed for 20 min., and 0·5 ml. of conc. HCl was then added. The volume was made up to 10 ml. with distilled water, and the sample was read in the colorimeter against standards similarly prepared from 1 ml. volumes of aqueous solutions of the appropriate compound, ranging in strength from 0 to 50 mg./100 ml.

Notes. Alkaline hydrolysis gave lower blanks from normal plasma than acid hydrolysis. No kaolin was added to the standards. A 1 cm. cell and a blue-green filter were used in the colorimeter.

Recoveries of compounds from plasma. Recoveries of known amounts of the compounds from plasma are shown in Table 1.

Table 1. *Recoveries of known amounts of compounds from plasma*

Compound*	Added (mg./100 ml.)	Found (mg./100 ml.)	Recovery (%)
I	5·0	5·5	110
	10·0	8·5	85
	20·0	22·0	110
	30·0	32·5	108
	50·0	46·5	93
II	10·0	10·4	104
	15·0	14·0	93
	20·0	21·7	108
	30·0	26·9	90
	40·0	38·5	96
	50·0	51·4	103
III	5·0	4·7	94
	10·0	9·1	91
	15·0	13·3	89
	20·0	18·3	91
	25·0	22·7	91
	30·0	23·9	80
	50·0	49·7	99
IV	5·0	5·5	110
	5·0	5·0	100
	15·0	13·5	90
	15·0	16·0	107
	25·0	22·5	90
	50·0	47·7	95
V	2·5	2·5	100
	5·0	5·5	110
	7·5	7·7	103
	12·5	12·5	100
	17·5	17·7	101
	25·0	23·8	95
VI	2·5	2·25	90
	2·5	2·5	100
	5·0	5·5	110
	7·5	8·2	109
	12·5	12·0	96
	15·0	15·7	105
	17·5	18·5	106
	17·5	18·5	106
	25·0	28·0	112
	25·0	23·0	92
VII	5·0	5·0	100
	15·0	17·0	113
	25·0	25·0	100
	25·0	26·1	104
	35·0	36·5	104
	50·0	53·5	107
VIII	10·0	15·0	150
	20·0	21·0	105
	30·0	29·5	98
	50·0	49·0	98
	70·0	66·0	94
	100·0	94·0	94
IX	5·0	5·0	100
	5·0	5·5	110
	10·0	10·5	105
	10·0	10·3	103
	15·0	15·0	100
	25·0	25·0	100
	25·0	27·0	108
	35·0	38·7	111
X	5·0	4·9	98
	10·0	9·7	97
	15·0	14·5	97
	20·0	18·4	92
	30·0	27·9	93
	50·0	49·5	99
XI	5·0	4·85	97
	10·0	12·1	121
	15·0	16·1	107
	20·0	20·8	104
	30·0	35·0	117
	40·0	45·2	113
XII	5·0	5·0	100
	10·0	8·6	86
	15·0	12·9	86
	20·0	15·2	76
	30·0	24·1	80
	50·0	43·5	87

* See p. 112.

Biological tests

Each compound was injected into a marginal ear vein of an adult rabbit in a dose of 100 mg./kg., as a 2·5, 5 or 10% solution of the sodium salt. Oxalated blood samples were taken at intervals from the marginal vein of the other ear, or by cardiac

puncture, and immediately centrifuged. The compound was then determined in the plasma by the appropriate method. From this preliminary experiment the approximate degree of persistence of the compound was calculated. It was then administered under the same conditions to a group of three rabbits, blood samples being withdrawn at standard intervals indicated by the preliminary experiment. From the plasma concentrations in the three rabbits a mean plasma concentration-time curve was constructed.

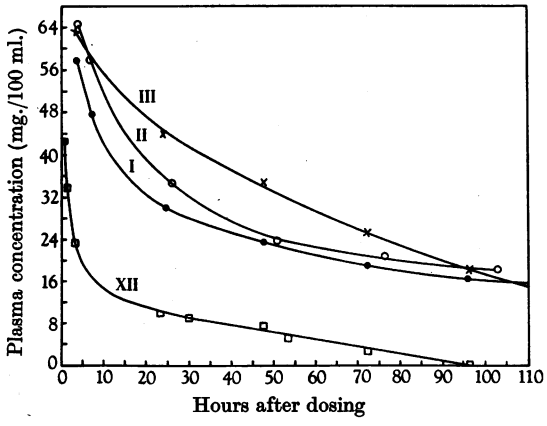


Fig. 1. Mean plasma concentration-time curves of compounds I, II, III and XII following the intravenous administration of 100 mg./kg. to groups of three rabbits.

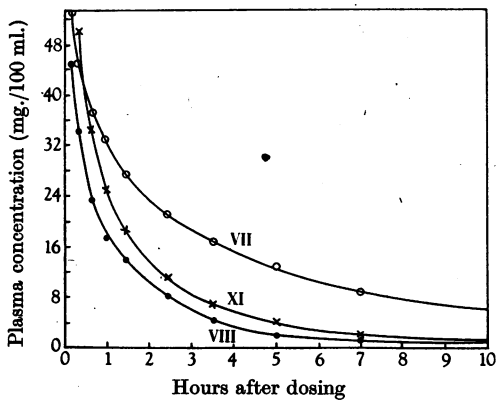


Fig. 2. Mean plasma concentration-time curves of compounds VII, VIII and XI following the intravenous administration of 100 mg./kg. to groups of three rabbits.

RESULTS

The mean plasma concentration-time curves of the twelve compounds are shown in Figs. 1–4. Since the compounds differed widely in persistence the scales of the four curves also differ widely, and should be carefully noted. The compounds are arranged in approximate order of decreasing persistence; this order is: I, II, III; XII; VII, XI, VIII; IV, IX, X, VI, V. The first group, including suramin (I), 'demethylated' suramin (II), and a closely related compound (III), having H acid as an end group instead of naphthylaminetrisulphonic acid, and sulphanilamide as a linking group instead of *m*-aminobenzamide, persists in the blood stream for extended periods. After 5 days all three compounds are still present in the plasma at concentrations of about

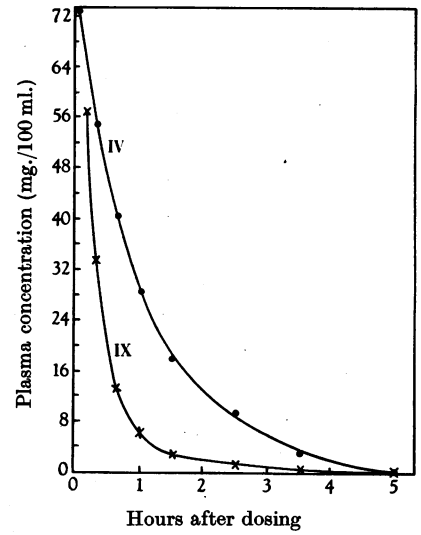


Fig. 3. Mean plasma concentration-time curves of compounds IV and IX following the intravenous administration of 100 mg./kg. to groups of three rabbits.

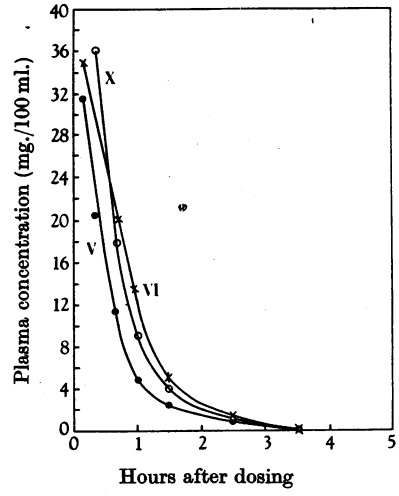


Fig. 4. Mean plasma concentration-time curves of compounds V, VI and X following the intravenous administration of 100 mg./kg. to groups of three rabbits.

16 mg./100 ml. The suramin curve is almost identical with that obtained by Dangerfield *et al.* (1938*b*) under similar conditions. The forms of the other two curves suggest that 'demethylated' suramin is very similar to the parent compound, and that the H acid derivative may be slightly less persistent. Compound XII, an analogue of suramin lacking two aminobenzoic acid links, is intermediate in persistence between the first group and the other, more rapidly eliminated, compounds. It is strongly persistent by ordinary standards, being detectable in the blood stream for 3–4 days. The third group of compounds (VII, VIII and XI) is rather weakly persistent, of about the same order as sulphadiazine (cf. Rose & Spinks, 1946). Each is detectable in the blood stream for 10–24 hr. The remaining five compounds (IV, V, VI, IX and X) are very rapidly eliminated, 1-naphthylamine-4:6:8-trisulphonic acid (IV) somewhat less so than the others. The results on IV, V and VI agree with those of Dewey & Wormall (1946) who examined a mixture of the three obtained by hydrolysis of suramin.

DISCUSSION

The results will be discussed in relation to the three problems outlined in the introduction.

1. *Persistence of suramin in relation to molecular structure*

The results show two main trends. First, compounds containing naphthalenepolysulphonic acids as end groups are more persistent than those containing carboxylic acids as end groups (cf. XI and XII; VII and IX; IV and V). Secondly, persistence tends to increase with increase in molecular weight (cf. XII and I, X and XI). From these two trends, and from individual characteristics of the twelve compounds, the following tentative deductions can be made. First, persistence of the suramin order is displayed only by polyamides containing 6 amino-acid groups (I, II, III as compared with XII). Secondly, high persistence is displayed only by polyamides containing naphthalenepolysulphonic acid groups (I, II, III, XII, VII, as compared with IX, X, XI). Thirdly, the presence of two naphthalenepolysulphonic acid groups appears essential for high persistence (XII as compared with VIII). Fourthly, *m*-amino-*p*-toluic, *m*-aminobenzoic and sulphanilic acid groups may be interchanged in the molecule without marked effect on the persistence (I, II, III). Fifthly, a phenolic group may be introduced without marked effect on the persistence (III). Sixthly, the shape and spacing of the molecule may be much modified without marked effect on the persistence (III).

All these deductions taken together strongly suggest that any symmetrical compound of high molecular weight containing a polysulphonic acid as an end group would show strong persistence. This view is supported by the rather strong persistence of some azo dyes of high molecular weight derived from naphthalenesulphonic acids (cf. Evans Blue, Trypan Blue; Dawson, Evans & Whipple, 1920). The susceptibility of the azo linkage to enzymic reduction is possibly what prevents their being retained in the body as long as suramin.

The fact that the spacing of the molecule does not appear to be of prime importance was not foreseen. It had been tentatively assumed that the persistence was due to the association of both polysulphonic acid groups with basic groups of a protein molecule. The difference in shape between I and III was therefore expected to result in reduced persistence of III. However, it may be that the two compounds combine with different proteins, or at different points of the same protein; and combination of compound with protein through the amide groups might also be possible. In that case, however, the dicarboxylic acid XI would be expected to show higher persistence.

2. *Persistence of suramin in relation to activity*

The low activity of II (Davey, 1943) and the trace activity of III (Browning, 1939), which are both of similar persistence to suramin, show that persistence and activity are due to different structural features of the molecule. The inactivity of many compounds prepared by Fourneau, Trèfouel, Trèfouel & Vallée (1924) which, from these results, would almost certainly be highly persistent, supports this view.

3. *Persistence of suramin in relation to its metabolism*

Compounds IV, V, VI, VII, VIII, IX, X, and XI are the chief potential hydrolysis products of suramin. Each is either very rapidly, or rather rapidly, eliminated. Other possible hydrolysis products, including asymmetrical ureas, would probably behave very similarly (cf. VIII, XI, XII). It can therefore be assumed that suramin either is not hydrolyzed *in vivo* or is hydrolyzed extremely slowly. The latter possibility appears rather unlikely, and the former may therefore be tentatively accepted. Other possible routes of metabolism could, however, be suggested which would probably give compounds of similar persistence to suramin, e.g. the oxidation of the latter to phenols, or phenolic sulphates or glucuronides. The long duration of the prophylactic action of suramin, and the known delicacy of the balance between structure and activity (Fourneau *et al.* 1924) strongly suggest that suramin is relatively resistant to metabolic processes.

SUMMARY

1. The persistence of suramin and eleven related compounds in the blood of rabbits has been investigated following their intravenous administration under standard conditions.

2. Marked persistence is a property of polyamides of high molecular weight that contain naphthylaminepolysulphonic acids as end groups.

3. Carboxylic acids of similar molecular complexity are more rapidly eliminated.

4. There is no relation between persistence and therapeutic activity.

5. Suramin is probably not hydrolyzed *in vivo*.

6. Methods of determining each of the twelve compounds in plasma are described.

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REFERENCES

Beyer, K. H. (1947). *Science*, **105**, 94.
Beyer, K. H., Peters, L., Woodward, R. & Verwey, W. F. (1944). *J. Pharmacol.* **82**, 310.
Boursnell, J. C., Dangerfield, W. G. & Wormall, A. (1939). *Biochem. J.* **33**, 81.
Boursnell, J. C. & Wormall, A. (1939). *Biochem. J.* **33**, 1191.
Browning, C. H. (1939). Private communication.
Brunelli, B. (1934). *Arch. farmacol. sper.* **57**, 186.
Collier, W. A. (1926). *Rev. sud-amer. Endocrin.* **9**, 179.
Collier, W. A. (1927). *Z. ges. exp. Med.* **54**, 606.
Dangerfield, W. G., Gaunt, W. E. & Wormall, A. (1938*a*). *Biochem. J.* **32**, 59.
Dangerfield, W. G., Gaunt, W. E. & Wormall, A. (1938*b*). *Biochem. J.* **32**, 65.
Davey, D. G. (1943). Private communication.
Dawson, A. B., Evans, H. M. & Whipple, G. H. (1920). *Amer. J. Physiol.* **51**, 232.
Dewey, H. M. & Wormall, A. (1946). *Biochem. J.* **40**, 119.
Earle, D. P. (1944). *J. clin. Invest.* **23**, 914.
Fisher, S. H., Troast, L., Waterhouse, A. & Shannon, J. A. (1943). *J. Pharmacol.* **79**, 373.
Fourneau, E., Trèfouel, J., Trèfouel, J. & Vallée, J. (1924). *Ann. Inst. Pasteur*, **38**, 81.
Gregerson, M. I. & Rawson, R. A. (1943). *Amer. J. Physiol.* **138**, 698.
Hawking, F. (1940*a*). *Trans. Roy. Soc. Trop. Med. Hyg.* **34**, 37.
Hawking, F. (1940*b*). *Trans. Roy. Soc. Trop. Med. Hyg.* **34**, 217.
Lang, K. (1931). *Arch. exp. Path. Pharmak.* **160**, 560.
Levaditi, C. & Klarenbeek, A. (1927). *C.R. Acad. Sci., Paris*, **184**, 1213.
Lundquist, F. (1945). *Acta Pharmacol. Toxicol.* **1**, 307.
Mandel, J. A. & Steudel, H. (1926). *Hoppe-Seyl. Z.* **160**, 91.
Mayer, M. (1922). *Arch. Schiffs- u. Tropenhyg.* **26**, 237.
Mayer, M. & Menk, W. (1922). *Arch. Schiffs- u. Tropenhyg.* **26**, 208.
Mayer, M. & Zeiss, H. (1920). *Arch. Schiffs- u. Tropenhyg.* **24**, 257.
Rantz, L. A., Kirby, W. M. M. & Randall, E. (1944). *J. clin. Invest.* **23**, 789.
Rawson, R. A. (1943). *Amer. J. Physiol.* **138**, 708.
Rose, F. L. & Bevan, H. G. L. (1944). *Biochem. J.* **38**, 116.
Rose, F. L. & Spinks, A. (1946). *J. Pharmacol.* **86**, 264.
Spinks, A. & Tottey, M. M. (1946). *Ann. trop. Med. Parasit.* **40**, 101.
Steppuhn, O. & Utkin-Ljubovzov, X. (1924). *Klin. Wschr.* **3**, 154.
Vierthaler, R. W. & Boselli, A. (1939). *Arch. Schiffs- u. Tropenhyg.* **43**, 149.
Zeiss, H. & Utkina-Ljubovtzova, K. (1930). *Z. Immun-Forsch.* **68**, 170.

The Action of Chymotrypsin and Trypsin on Insulin

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Of the various chemical methods used in attempts to elucidate the structure of proteins, the breakdown by mild hydrolysis seems most promising. The constitution of the resulting fragments which can be isolated may then be ascertained separately. This method has been used to some extent by Gordon, Martin & Synge (1941, 1943) who identified a considerable number of small peptide fragments after partial hydrolysis with hydrochloric acid. Whilst the method undoubtedly throws some light on the order of amino-acids in the protein, a complete structural analysis would be a formidable, if not impossible, task, although it is capable of elucidating the structure of the pentapeptide gramicidin S (Synge, 1944, 1945; Consden, Gordon, Martin & Synge, 1946) and is no doubt capable of considerable extension. A more promising method in the case of a protein is hydrolysis by proteolytic enzymes into larger peptides which can then be further examined.