REFERENCES

1 C. W. PARKER, I. SHAPIRO, M. KERN and H. N. EISEN, *J. exp. med.* **115**, 821 (1962).
2. A. L. WECK and N. H. EISEN, *J. exp. med.* **112**, 1227 (1960).
3. M. ROLLE and H. MAYER, *Arch. Hyg. Bakt.* **137**, 596 (1954).
4. L. P. GARROD, *Experimental Chemotherapy*, 2nd edn. vol. 2, p. 1. Academic Press, New York (1964).

Biochemical Pharmacology, Vol. 16, pp. 1367–1370. Pergamon Press Ltd. 1967. Printed in Great Britain

Studies on the role of the liver component of reticulo-endothelial system (RES) in the metabolism of sulfametoxipiridazine and sulfametoxipirimidine

(*Received* 12 *September* 1966; *accepted* 23 *January* 1967)

THE long acting sulfonamides are of great importance in therapeutics but their secondary toxic effects have to be guarded against in their clinical applications.[1–6] Knowing the stress to which the RES undergoes in pathological processes in which the sulfonamidetherapy is applied, we undertook to study this morpho-functional system as related to the metabolism of two long acting sulfonamides; sulfametoxipiridazine (LD_{50} for mice, applied per os 3·5 g/kg body weight) and sulfametoxipirimidine (LD_{50} for mice, applied per os, 16 g/kg body weight) with different toxicities.

420 H-strain, male mice, weighing 18–22 g were divided into groups of 5–20 animals each. The sulfonamides were applied orally in single doses of 1 g/kg body weight. At various intervals after administration of the sulfonamides (2, 4, 8 hr), the mice were killed by the sectioning of the art. carotidis, and samples of blood, liver and kidney collected for determinations. The amounts of free and bound sulfonamides was assayed by the Bratton–Marshall method.[8]

To modify the functional capacity of liver component of the RES each animal was injected with 0·2 ml of a 10 per cent colloidal India ink solution,[9, 10, 11] 48 hr before the administration of the sulfonamides, and for 5 days, previous to the administration of the sulfonamides, with daily subcutaneous 0·2 ml doses of a Reticulin solution of the peptide type.[12] The substance stimulates the functions of the reticulo-endothelial tissue, prevents and reduces the increased permeability of the capilars, determinated by histamine. The animals were subjected to a combined treatment with these substances, both to establish the concentrations of sulfonamide in the tissues, and to follow the toxicity for a period of 28 days.

The single dose of sulfametoxipiridazine (1 g/kg body weight) does not produce, over a period of 28 days, any lethality in mice (Fig. 1). In animals submitted to a previous treatment with India ink the

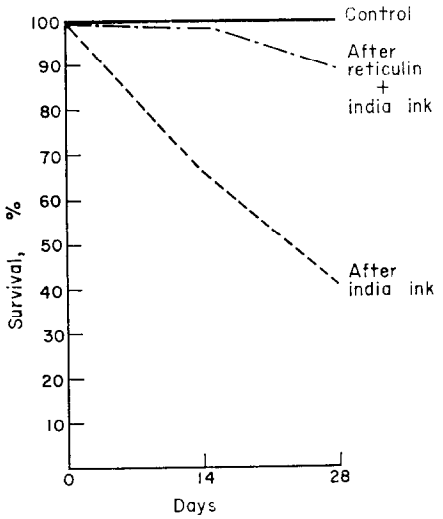

FIG. 1. Toxicity of 1g/kg per os sulfametoxipiridazine (40 animals per point).

same single dose provokes a mean lethality of 60 per cent (Fig. 1). In animals pretreated over a period of 5 days with doses of Reticulin, and subsequently with India ink, after the administration of sulfametoxipiridazine, lethality is only 11 per cent. The single dose of sulfametoxipirimidine (1 g/kg body weight) does not provoke, over a period of 28 days any lethality in mice (Fig. 2). The same dose given to animals pretreated with India ink produces a mean lethality of 35 per cent, and one of 57 per cent in mice treated for 5 days with Reticulin and then with India ink, over the same period (Fig. 2).

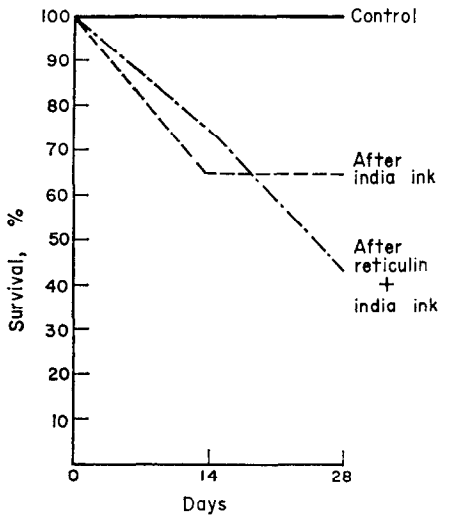

FIG. 2. Toxicity of 1g/kg per os sulfametoxipirimidine (40 animals per point).

By using the same procedure in other groups of animals, the amount of free and bound sulfonamide (acetylated forms of the sulfonamide in position 1 and 4) was measured in blood, liver and kidney, at 8 hr after administration of the single dose (1 g/kg body weight) of sulfonamide. Figs. 3 and 4

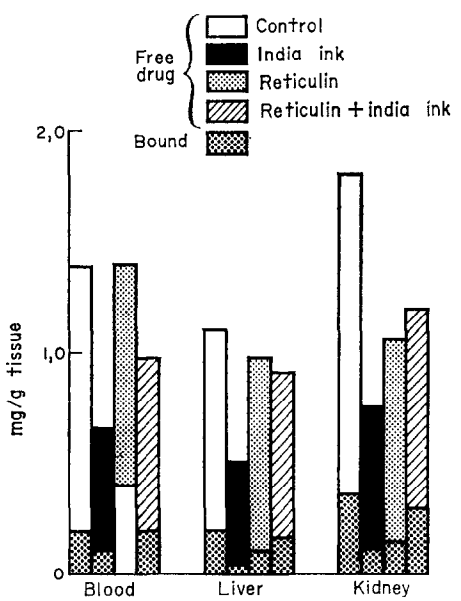

FIG. 3. Tissue concentration of sulfametoxipiridazine (20 mice for each series).

show that there are differences between the concentrations in the tissues, of the free and the bound sulfonamide after a previous treatment with India ink and/or Reticulin.

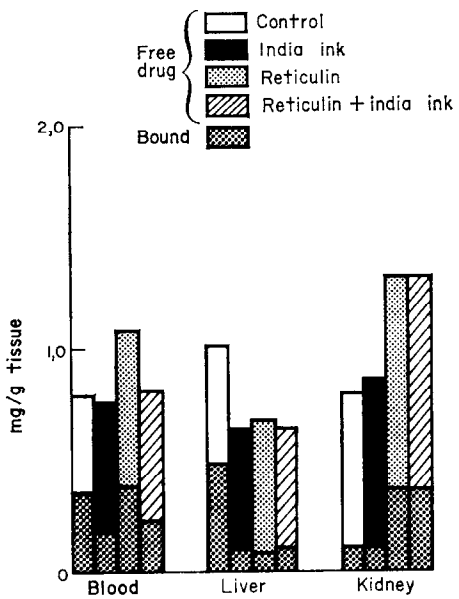

FIG. 4. Tissue concentration of sulfametoxipirimidine (20 mice per each series).

From the findings shown in Figs. 1–4 it can be seen that the single dose of 1 g/kg body weight of sulfametoxipiridazine or sulfametoxipirimidine, does not produce, under normal conditions, any lethality over a period of 28 days. The functional modification of the hepatic RES leads, for the same dose, to a certain lethality. It is of interest that while under the influence of Reticulin lethality decreases following sulfametoxipiridazine, it increases—under the same conditions—for sulfametoxipirimidine. This difference has been ascribed by us to the fact that the two sulfonamides have different metabolic pathways.[7] In the organism sulfametoxipiridazine is acetylated while suflametoxipirimidine is converted by glucuronconjugation; the acetylation of the sulfonamides is accelerated by the hepatic RES.[13] The findings in Figs. 3 and 4 support the hypothesis that the RES interferes with these processes in the metabolism. Reticulin only stimulates acetylation and this explains why it effects only sulfametoxipiridazine.

Since a sulfonamide like sulfametoxipirimidine, considered to have but a small toxicity (16 g/kg body weight), may have toxic effects under conditions in which it acts upon a damaged RES, the problem of preventing the secondary effects in the therapy with long acting sulfonamides is open to further studies.

Pharmacological Department,
Chemical-Pharmaceutical Research Institute,
Bucarest 4,
Rumania

M. TIMAR
A. BOTEZ
M. TEODORESCU
I. HÄDRICH
I. NITELEA

REFERENCES

1. H. PÉQUIGNOT and P. EVEN, *Produits probl. pharm.* **19**, 229 (1964).
2. H. WITZGALL, H. BOYENS, *Archs int. Pharmacodyn. Thér.* **148**, 511 (1964).

3. H. KUMMERLE, *Klinik und Therapie der Nebenwirkungen* G. Thieme, Stuttgart (1960).
4. G. SALVI and A. C. PLANCKER, *Farmaco*, **2**, 75 (1964).
5. J. HAZARD, *Thérapie* **1**, 33 (1963).
6. H. HUTCHINSON, *J. Am. med. Ass.* **185**, 243 (1963).
7. *Experimental Chemotherapy*, vol. II. Academic Press (1964).
8. C. BRATTON and E. MARSHALL, *J. biol. Chem.* **128**, 137 (1939).
9. J. VANLERENBERGHE, L. ADEINS, R. GUISLAIN and C. BEL, *J. Physiol., Paris*, **17**, 784 (1965).
10. *Colloques Cent. Nat. Rech. scient.* sur SRE. Ed. Masson (1963).
11. N. JANCSÓ, *Speicherung, Stoffanreicherung im Retikuloendothel und in der Niere.* Akadémia, Budapest (1955).
12. I. MOLDOVAN, *Anal. Acad. rom.* Ser. III, **13**, 6 (1948).
13. W. C. GOVIER, *J. Pharmac. exp. Ther.* **150** 305 (1965).

Biochemical Pharmacology, Vol. 16, pp. 1370–1374. Pergamon Press Ltd. 1967. Printed in Great Britain

Microsomal ATPase of rabbit brain and effects of general anesthetics

(*Received* 5 *November* 1966; *accepted* 20 *December* 1966)

IN RECENT years an interaction of Na^+- and K^+-activated, Mg^{2+}-dependent adenosine triphosphatase (Na^+K^+-ATPase) and cation translocation at excitable cell membranes has been well established.[1] The present study was indertaken to investigate a possible effect of general anesthetics on ouabain-sensitive Na^+K^+-ATPase of microsomal fractoins of rabbit brain.

Cerebral microsomal fraction of rabbit were prepared according to the method of Skou.[2] Rabbits were sacrificed by injection of air into an ear vein and the brain rapidly removed and chilled in iced 0·25 M sucrose with 0·03 M histidine buffer. After removal of the brain stem, the cerebrum was homogenized in a Potter–Elvehjem glass homogenizer fitted with a Teflon pestle, with 7 vol. of 0·25 M sucrose containing 0·03 M histidine, 0·005 M EDTA, and 0·1% deoxycholic acid, adjusted to pH 6·8 with Tris buffer. After centrifugation at 8000 *g* for 15 min, the supernatant fraction was again centrifuged in a Hitachi 40-P ultracentrifuge* at 59,300 *g* for 1 hr at 0°. The packed sediment was resuspended in half the original volume of histidine buffer containing 0·25 M sucrose, 0·03 M histidine base, and 0·001 M EDTA free of sodium, and the pH adjusted to 6·8 with Tris. The microsomal fraction was divided into small quantities and kept frozen until use. Significant loss of activity was not observed after several weeks, when the preparation was stored at −20°. Thawed material was used in the experiments.

The ATPase activity was measured in 1·2 ml of buffer solution with appropriate concentration of Tris-ATP and $MgCl_2$ and with or without 100 mM NaCl and 20 mM KCl; pH was again adjusted to 7·6 with Tris buffer. Sodium-free Tris-ATP was prepared by passing disodium ATP (Calbiochem) through a column of Amberlite IR 120 cation-exchange resin. After warming the ATP-containing solution in a glass-stoppered test tube for 5 min at 38° in a Dubnoff shaker, 100 μl of the microsomal fraction was introduced. The reaction was stopped at an appropriate time by addition of trichloroacetic acid.

Liberated inorganic phosphate was determined by a modification of the method of Fiske and SubbaRow,[3] which consisted of extraction of inorganic phosphate into isobutanol; ascorbic acid was used as the reducing agent. Activity was expressed as inorganic phosphate liberated per unit time, per 1 mg enzymatic protein. Enzymatic protein was determined by the biurett method described by Layne.[4]

Hydrolysis of ADP was determined in a similar fashion, replacing ATP with ADP. ADP (Sigma) was used as substrate without further purification. The activity was measured in the presence of Na^+ and K^+. A possible contamination of the preparation with adenylate kinase was determined

* Hitachi Co. Ltd., Tokyo, Japan.