For analytical results, see Table II. Taking into consideration the number of analyses, a non-parametric statistical method, the Mann-Whitney U Test, was used in the treatment of the data[10]. It is evident that there is no significant difference between the total activities of acid phosphatases in the sediment of the control group and the experimental group. This would seem to mean that the phosphatases are not inhibited by the sulfonamide or its metabolites. However, it must be stressed here that this conclusion is valid only if the production of lysosomes or lysosomal enzymes is not stimulated by the drug. Another prerequisite is that the composition of the liver is not changed. Against the assumption of an elevated enzyme production, it may be stated that sulfapyridine inhibits the production of thyroxine[11,12] and thereby probably also indirectly the synthesis of proteins, regulated by the hormone[13]. Concerning the composition of the liver and, indirectly, the choice of the wet weight of the liver as a reference, it is interesting to compare the composition of the livers of normal rats and starved rats. To judge from the analyses of rat livers made by FENN[14], it is evident that the reduced intake of food by the experimental group is of very little importance, if the wet weight is used as a reference.

Table I. Body weight and intake of food

		Body weight (g) mean values		Intake of food per day (g) mean values				
Group	No. of animals	Initial weight	Final weight	Day 1	Day 2	Day 3	Day 4	Day 5
Control	6	138	175	19.9	19.3	20.1	21.1	18.3[a]
Experimental	6	130	128	7.9	8.9	10.4	10.7	8.5[a]

[a] This consumption corresponds to an intake for 16 h only.

Table II. Activity of acid phosphatases in control and in experimental group

Group	No. of animals	Total activity of phosphatases/ml of resuspended sediment µmol P/ml/ 10 min	Released activity of phosphatases in % of total sedimental activity	Activity of phosphatases/ml 'Supernatant 2' µmol P/ml/ 10 min
Control	6	9.9 ± 0.32[a]	29 ± 4.2	1.3 ± 0.12
Experimental	6	9.3 ± 0.99	19 ± 1.5	1.8 ± 0.05
p[b]		> 0.39	0.002	0.002

[a] Mean $\pm$ S.D. [b] The p values were obtained in the non-parametric statistical analyses (Mann-Whitney U Test) of the differences between the 2 groups.

The acid-phosphatase activity of the 'Supernatant 2' of the treated animals shows a significantly higher value than that of the control group. An analysis of the individual values of the experimental group reveals that the activity of 'Supernatant 2' is fairly constant, irrespective of variations in the sedimental activity. Furthermore, calculations based upon the activities and the volumes of resuspended sediment and of 'Supernatant 2' (11 ml and 25 ml, respectively) show that the total activity of these cell fractions of the experimental group is 5% greater than that of the control group. It seems likely that the increase in activity of 'Supernatant 2' is due mainly to an extraneous source, probably erythrocytes, since sulfonamides are known to be hemolytic[15].

After incubation one finds that the released phosphatase activity of the sediment expressed as a percentage of the total sedimental activity is lower in the sulfapyridine-treated group of animals than in the control group. The decrease is 34%. This fact may mean that the decreased release of enzymes from the lysosomes is due to a direct stabilizing effect of sulfapyridine or its metabolites. This interpretation must, however, be advanced with caution, because sulfapyridine may exert its effect indirectly. The results obtained suggest further similar experiments with other sulfonamides and also in vitro experiments with the metabolites of the drugs, in order to get a clear relationship between drug and effect.

Zusammenfassung. Sulfapyridin, das unter anderem bei der Behandlung von Dermatitis herpetiformis benutzt wird, wurde Ratten per os gegeben. Eine 34%ige Senkung der Freisetzung von sauren Phosphatasen der Leberlysosomen wurde in vitro bei einem Vergleich mit einer Kontrollgruppe erhalten.

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[10] S. SIEGEL, in *Nonparametric Statistic for the Behavioral Sciences* (McGraw-Hill Book Co. Inc., New York, Toronto, London 1956), p. 116.
[11] C. G. MACKENZIE and J. B. MACKENZIE, Endocrinology *32*, 185 (1943).
[12] C. D. TURNER, in *General Endocrinology* (W. B. Saunders Co., Philadelphia and London 1960, reprint 1961), p. 123.
[13] J. R. TATA and C. C. WIDNELL, Biochem. J. *98*, 604 (1966).
[14] W. O. FENN, J. biol. Chem. *128*, 297 (1939).
[15] J. DAUSSET and L. CONTU, A. Rev. Med. *18*, 55 (1967).
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Susceptibility to Strychnine Convulsions in Maturing Rats

One of the several studies of ontogenesis in young rodents of seizure responses to various convulsant drugs has demonstrated a progressive increase in median convulsive dose (CD_{50}) for intraperitoneal strychnine in rats after the eighth day of life[1]. This change was attributed to a diminishing permeability of the central nervous system to the drug, although some other researches[2-4] question whether significant postnatal maturation of blood-brain barrier occurs in the rat. KATO et al.[5,6] later

[1] O. O. PYLKKO and D. M. WOODBURY, J. Pharmac. exp. Ther. *131*, 185 (1961).
[2] F. M. GRAZER and C. D. CLEMENTE, Proc. Soc. exp. Biol. Med. *94*, 758 (1957).
[3] J. W. MILLEN and A. HESS, Brain *81*, 248 (1958).
[4] A. SHIMODA, Acta path. jap. *95*, 13 (1963).
[5] R. KATO, E. CHIESARA and P. VASSANELLI, Jap. J. Pharmac. *12*, 26 (1962).
[6] R. KATO, P. VASSANELLI, G. FRONTINO and E. CHIESARA, Biochem. Pharmac. *13*, 1037 (1964).

reported that liver microsomal preparations from newborn rats showed only a slight capacity to metabolize strychnine in vitro, and that this capacity developed progressively through the first 30–50 days of life. These findings led us to undertake experiments to confirm the reported maturational decrease in sensitivity to strychnine, while also testing the hypothesis that such change could be attributed to an increase in capacity for strychnine inactivation.

Materials and methods. CD_{50}'s to strychnine sulfate in aqueous solution were determined in mature rats (6 months old) and in young rats (Holtzman strain) at 3, 4 and 5 weeks. The latter ages were chosen to correspond to the interval of 21–36 days over which the greatest relative increase in strychnine CD_{50} was found by PYLKKO and WOODBURY[1]. Three treatment methods were as follows: (a) intraperitoneal (i.p.) administration; (b) i.p. administration 50 min after previous i.p. injection of SKF 525A (50 mg/kg), an inhibitor of hepatic drug metabolism; (c) intravenous (i.v.) administration via the caudal vein.

Each CD_{50} determination was based on 40 rats. As we have found no sex difference in response at 3 and 4 weeks, CD_{50} determinations at those ages included both sexes. However, a significant sex difference beyond 4 weeks required utilization thereafter of data only from males in order that results might correspond to those of the previous study[1]. After graphical plotting of results, CD_{50}'s and 95% confidence limits were determined by the method of LITCHFIELD and WILCOXON[7]. Statistical comparisons between CD_{50}'s were by no means of potency ratios (and 95% confidence limits) calculated by the same method.

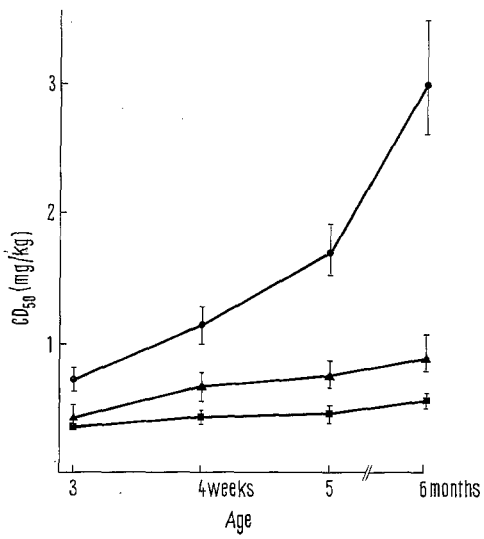
A possible direct influence of SKF 525A on convulsive susceptibility, suggested by a report of CNS stimulation in cats[8], was tested by determining the response to flurothyl for 2 groups of 15 rats after pretreatment with saline or with SKF 525A at the same interval and dosage indicated above. Such determinations were run on 35- to 36-day-old rats by the method of WEBB and DAVIS[9].

Results and discussion. Preliminary data from 2 groups of twelve 33- to 35-day-old rats, 1 pretreated with saline and 1 with SKF 525A, pointed to an important role of hepatic metabolism in responsiveness to i.p. strychnine. While 11 out of 12 rats convulsed after 1.6 mg/kg of strychnine alone, only 3 of 12 responded to the combined drug treatment (probability of difference < 0.005). In this and later tests utilizing the i.p. route, the latency of convulsions was 6–10 min after injection, while it was only 10–12 sec after i.v. infusion.

CD_{50}'s determined at the several ages are shown in the Figure. There was no significant deviation from parallelism among the regression lines for lethality. Statistical comparisons between all treatments at one age and within each treatment for different ages revealed those values which differ significantly ($p < 0.05$) as shown in the Table.

CD_{50}'s for i.p. strychnine at 3 and 5 weeks or 6 months were nearly identical to those of PYLKKO and WOODBURY at closest corresponding times, with a large increase between each of the successive ages of testing; therefore, their results are clearly confirmed. However, only a much smaller increase in the i.v. CD_{50} occurred over the total age span. The ratio i.p.-CD_{50}/i.v.-CD_{50} changed from 1.92 at 3 weeks of age to 5.15 at 6 months. GAINES et al.[10] and NATOFF[11] have both concluded recently that this sort of ratio measures the degree of hepatic inactivation occurring in the case of cholinesterase inhibitors. Therefore, it appears that the age-related increase of this ratio for strychnine (which results almost entirely from the age-related elevation of i.p.-CD_{50}) reflects almost entirely an age-dependent increase in the capacity for strychnine metabolism by the liver.

The comparison of CD_{50}'s for i.p. strychnine after SKF 525A to those after i.p. strychnine alone shows a similar age-related increase in the ratio: i.p.-CD_{50}/i.p.-



Median convulsive doses in mg/kg for strychnine i.p. (●——●), strychnine i.p. after 50 mg/kg SKF 525A (▲——▲), and strychnine i.v. (■——■), in maturing rats.

Comparisons among strychnine median convulsive dose values for difference ages and modes of administration. Results of statistical comparisons

Treatment	3 vs 4 weeks	4 vs 5 weeks	5 weeks vs 6 months
(a) I.p. strychnine	[a]	[a]	[a]
(b) I.p. strychnine after SKF 525A	[a]	NSD	NSD
(c) I.v. strychnine	[a]	NSD	[a]
Age	**(a) vs (b)**	**(b) vs (c)**	**(a) vs (c)**
3 weeks	[a]	NSD	[a]
4 weeks	[a]	[a]	[a]
5 weeks	[a]	[a]	[a]
6 months	[a]	[a]	[a]

[a] Significant difference, $p < 0.05$.
NSD, no significant difference, $p > 0.05$.

[7] J. T. LITCHFIELD JR. and F. WILCOXON, J. Pharmac. exp. Ther. *96*, 99 (1949).
[8] B. B. GAITONDE and H. L. BORISON, Toxic. appl. Pharmac. *8*, 118 (1966).
[9] O. L. WEBB and W. M. DAVIS, Archs int. Pharmacodyn. Ther. *150*, 177 (1963).
[10] T. B. GAINES, W. J. HAYES JR. and R. E. LINDER, Nature *209*, 88 (1966).
[11] I. L. NATOFF, J. Pharm. Pharmac. *19*, 612 (1967).

CD_{50} after SKF 525A, from 1.74 at 3 weeks to 3.30 at 6 months. Unless one assumes a change with age in the accessibility or the susceptibility of hepatic enzymes to SKF 525A without any evidence suggesting these alternatives, the best interpretation of the ratio data would again be that of an age-dependent increase in hepatic capacity for strychnine inactivation. These data again point to the interpretation of i.p. strychnine CD_{50} changes with age as results of postnatal ontogenesis of hepatic enzyme activity, as was suggested also by the data of KATO et al.[5,6].

By its chemical nature the volatile convulsant flurothyl is insensitive to metabolic inactivation. The maximal (tonic-clonic) flurothyl response, which corresponds most nearly to the strychnine seizure, was not significantly facilitated by SKF 525A pretreatment. Furthermore, the intensity of response to flurothyl was decreased rather than increased by pretreatment. Therefore, it is not possible to attribute SKF 525A effects on strychnine CD_{50}'s to a direct CNS action.

The relatively slight increase in CD_{50}'s with age in our groups receiving i.v. strychnine and i.p. strychnine plus SKF 525A does not allow the total exclusion of maturational changes in the CNS such as that of the blood-brain barrier postulated by PYLKKO and WOODBURY[1]. However, the data indicate that such events, if occurring after 3 weeks of age, have a much smaller effect on sensitivity to strychnine than was suggested by the results following i.p. injection in the former study.

Résumé. Chez le rat ce n'est pas la perméabilité du système nerveux central, mais le métabolisme du foie qui joue le rôle principal dans la susceptibilité á la strychnine inoculée par voie intrapéritonéale.

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Na-Activated Calcium Efflux in Rabbit Vagus Nerve Fibres

A system which exchanges intracellular calcium against extracellular sodium has recently been described in mammalian heart[1] and squid giant axons[2]. It seems that extrusion of calcium is driven by influx of sodium along a concentration gradient; a common carrier has been postulated[1] for the outward movement of calcium and part of sodium entry. Evidence from the present paper suggests the existence of a similar system in mammalian nerve fibres. The experiments were performed on rabbit vagus nerves, which consist mainly of non-myelinated fibres[3] and thus are particularly suitable for ion-exchange-studies.

Desheathed vagus nerves were incubated for 2 h at 37 °C in Locke's solution containing tracer amounts of radioactive calcium. After rinsing for 20–30 sec in tracer-free medium, the ends of the nerves were covered with vaseline, fixed in a small tube (volume 0.35 ml) and the preparations washed by a constant flow (1 ml/min) of bathing solution. The composition of this solution could be changed rapidly by switching to another reservoir. Washing fluid fractions of 3 ml were collected in counting vials and, after addition of 2 drops of 1 *M* oxalate, dried at 90 °C. At the end of the experiment, the nerves were homogenized in 6 ml Locke's, transferred to a counting vial and dried. 3 ml of scintillator fluid (250 mg POPOP, 2 g PPO, 1000 ml toluene) was added to the dry samples and radioactivity was determined in a Packard Tricarb scintillation counter. Different amounts of ^{45}Ca comparable to those released by the nerve during a 3 min period proved to be perfectly measurable under these conditions. Since self-absorption varied with certain solutions, it was necessary to establish correction factors for quenching. Alternatively, in the experiments related to the effect of temperature on Ca-efflux, the nerves were successively immersed in a series of vials containing 3 ml Locke's. Radiocalcium was then measured as above. From the amounts of ^{45}Ca found in the samples, the rate-constant for calcium loss was calculated and plotted on semilogarithmic paper. Locke's solution was of the following composition (m*M*): NaCl 153, KCl 5.6, $MgCl_2$ 0.5, $CaCl_2$ 2.2, *tris* 1, glucose 5.5. In several experiments Na^+ of the solution was replaced by equimolar amounts of Li^+ or choline.

The curve represented in Figure 1 results from a standard washout experiment at 21 °C and shows the rate-constant of Ca-efflux as a function of time. A similar result has been obtained by KEYNES and RITCHIE[3], who applied drugs and electrical stimulation to rabbit vagus nerves during the second hour of calcium washout. In the present experiments, however, solutions which were to be examined for their effect on calcium loss were applied from min 24 to min 60 after beginning of the experiment. The curve is far from being exponential in this region, but the interval was chosen on the assumption that this part of outflux comes preferably from the easily accessible C-fibres.

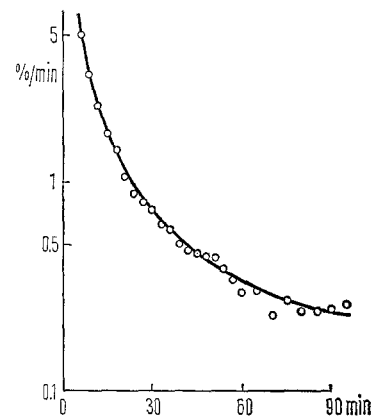

Fig. 1. Efflux of Ca from desheathed rabbit vagus nerve as a function of time. Temperature 21 °C.

[1] H. REUTER and N. SEITZ, J. Physiol. *195*, 451 (1968).
[2] M. P. BLAUSTEIN and A. L. HODGKIN, J. Physiol. *200*, 497 (1969).
[3] R. D. KEYNES and J. M. RITCHIE, J. Physiol. *179*, 333 (1965).