lengthwise extension of growing fibers is also affected by constriction, remains to be determined; if it is, the puzzling fact that the rate of advance of nerve fibers inside the distal stump is faster after crushing (precluding scars) than after suture (with some scar formation),[8] would find a ready explanation.

In practical regards, the demonstration of the additional hazard of scar contraction only underlines the value of measures designed to eliminate scar formation. In saying this, we take the desirability of complete caliber recovery and myelinization for granted, although their relevance for functional recovery has never been accurately determined.

Summary. Nerve fibers regenerating in a nerve with localized constriction are greatly reduced in caliber and delayed in myelinization at all levels distal to the constriction. Scar tissue, therefore, presents not only a local impediment to the outgrowth of regenerating fibers, but a continued hazard to their maturation.

[8] Gutmann, E. J., *Neurol. and Psych.*, 1942, **5**, 81.

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Acute Toxicity of Mercurial Diuretics.

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The present experiments were performed to compare the acute toxicity of the mercurial diuretics, and to study the effect of theophylline and the speed of intravenous injection upon the acute toxicity.

Cats were used as test animals. Eight materials were studied: (1) mercuric chloride, (2) mercuric chloride mixed with theophylline, (3) mercurin, (4) mercupurin (of commerce), (5) salyrgan, (6) salyrgan-theophylline (of commerce), (7) salyrgan freshly mixed with theophylline, (8) same as (7) but allowed to age for a month at room temperature. The acute toxicity was determined by the amount of mercury necessary to cause death in unanesthetized cats by frequent, regularly spaced intravenous injections.

Results. The results are summarized in Table I. They show that the acute toxicity of mercury is essentially the same whether administered in the form of mercuric chloride, mercuric chloride with theophylline, or salyrgan; namely, the fatal dose is approximately 15 mg Hg per kg.

However, mercury is only about one-half as toxic when administered in the form of salyrgan-theophylline, mercupurin, and mercurin; *i.e.*, the fatal dose is about 30 mg Hg per kg. These results fail to confirm those of DeGraff and Lehman[1] who found that the toxicity increased in the order mentioned.

It is clear that theophylline does not alter the toxicity of all mercurials since the mercupurin which contains the theophylline has the same toxicity as the mercurin which is without theophylline, and the mercury in mercuric chloride has the same toxicity with or without theophylline.

In the case of salyrgan, however, theophylline does alter the toxicity. It reduces the toxicity to one-half. Such an influence of theophylline was recently reported by DeGraff and Lehman[1] who ascribed the change to a chemical reaction which occurred when the 2 drugs were mixed. Our results also favor some form of reaction between them because time is required before the mixture will exhibit lower toxicity than the salyrgan alone. When the test was made directly after the 2 compounds, salyrgan and theophylline were mixed in our laboratory, the toxicity of the mercury was the same as that of salyrgan alone. However, when the mixture was allowed to stand for a period at room temperature, its toxicity was reduced to

[1] DeGraff, A. C., and Lehman, R. A., *J. A. M. A.*, 1942, **119**, 998.

TABLE I.
Lethal Dose of Mercurial Diuretics in Cats.

Rate of injection	$HgCl_2$ 0.1%	$HgCl_2$ 0.1% and Theophylline 0.05%†	Mercurin	Mercupurin	Salyrgan	Salyrgan-Theophylline (commercial preparation)	Salyrgan mixed with Theophylline same proportions as commercial preparation: freshly mixed	Salyrgan mixed with Theophylline same proportions as commercial preparation: one month old
0.4 mg Hg/kg/min.	24.8	12.0	12.8	12.8	11.2	30.1		
	15.9	11.6	22.5	13.6	22.3	37.8		
	21.8	31.3	24.8	37.6	7.9	25.6		
	15.9	22.6	40.2	34.3	12.6	33.9		
	7.7	12.7	55.3	45.0	28.1	31.2		
Avg	17.2	18.0	31.1	28.7	16.4	31.7		
S.E.	±2.9	±3.9	±6.1	±6.5	±3.8	±2.0		
0.8 mg Hg/kg/min.	9.6		31.0	40.0	21.0	37.4		19.2
	12.8		31.4	33.9	14.4	44.3		28.8
	12.8		24.2	11.3	12.6	28.5		44.8
	19.2		7.9	11.3	11.4	40.5		35.2
	30.4		33.4	35.0	8.3	30.6		19.2
Avg	17.0		25.6	26.3	13.5	36.3	14.8*	29.4
S.E.	±3.7		±4.7	±6.2	±2.1	±3.0	±1.6	±4.9
1.6 mg Hg/kg/min.	20.4	10.2	34.9	12.8	9.9	7.9		
	10.2	13.6	45.1	35.4	13.0	17.7		
	22.3	10.2	41.3	35.2	9.4	32.0		
	6.8	21.8	12.8	41.3	8.0	22.1		
	6.8	13.6	28.8	38.4	29.1	36.2		
Avg	13.3	13.9	32.6	32.6	13.9	23.2		
S.E.	±3.4	±2.1	±5.7	±5.1	±3.9	±5.1		
Grand Avg	15.8	16.0	29.8	29.2	14.6	30.3	14.8	29.4
S.E.	±1.9	±2.2	±3.3	±3.3	±1.8	±2.4	±1.6	±4.9

Each figure represents one cat. All values are expressed in terms of mg Hg per kg. Concentration of elemental mercury in all the organic preparations as diluted for injection is the same: 1.6 mg Hg per cc.

* This average figure was obtained on 10 cats, injected at the rate of 0.8 mg Hg per kg per min., dying of 20.8; 21.1; 16.0; 9.6; 11.4; 8.0; 9.6; 14.4; 20.8; and 16.0 mg Hg per kg, respectively.

† Theophylline ethylenediamine was used.

that of the preparation of commerce, salyrgan-theophylline. No visible changes in the solution occur on standing. We have no data on the rate at which the change takes place. In view of the influence of aging, it will be necessary to test other mercurials in that way before the matter of the effect of theophylline on toxicity is decided.

In relation to the effect of the speed of injection on toxicity, DeGraff and Lehman[1] observed that the lethal dose was smaller the slower the rate of injection. Such a phenomenon is often seen in the case of drugs which develop their actions slowly so that in the course of rapid injection one may overrun and secure a value which is larger than the true minimal lethal dose. That being the case, one may question the inference which the authors made that the drug is less toxic when given rapidly. It may also be noted that our results fail to show any significant or consistent difference in the lethal dose within wide variations in the rate of administration of the mercurials examined. In the study by DeGraff and Lehman,[1] the rates of injection varied from 1 to 10 mg Hg per kg per minute, so that death was produced in from 3 to 7.5 minutes in the group receiving the mercupurin

most rapidly. In our study, the mercurial was injected far more slowly, from 0.4 to 1.6 mg Hg per kg per minute. Values approximating ours for the lethal dose of mercupurin, mercurin, salyrgan, and salyrgan-theophylline were obtained by DeGraff and Lehman,[1] when they injected the mercurial slowly (1 mg Hg per kg per minute). This favors the conclusion that in their titrations the actual acute lethal dose was overrun, in the case of their rapid injections, in proportion to the speed of administration.

Conclusions. (1) The admixture of theophylline with salyrgan reduces the toxicity of the latter as the result of some reaction between the two compounds inasmuch as aging is required before the changed toxicity is demonstrable. (2) This influence of theophylline did not apply to mercurin. (3) In terms of mercury, non-ionizable organic mercurials are not necessarily less toxic than mercuric chloride.

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Cultivation of *Trypanosoma gambiense in vitro* in Cell-Free Medium.*

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The diagnosis of African Sleeping Sickness has depended upon confirming clinical impressions by a direct demonstration of trypanosomes in the blood, lymph node fluid, or spinal fluid of the patient or else indirectly by animal inoculation. It seems that many infected cases might be overlooked by these methods, a particularly unfortunate circumstance in the early stages of the disease when treatment is usually so very effective.

In this preliminary paper it will be shown that it is easy to cultivate *Trypanosoma gambiense*, and that by this means infection may be demonstrated when other methods fail. Amongst previous investigators of this problem there is space here to mention only Brutsaert and Henrard[1] whose brilliant and successful work with cultures at Leopoldville, Belgian Congo, was a marked stimulus.

Two strains of *Trypanosoma gambiense*, both originating in Liberia, were used.† Both behave similarly in cultures.

The culture medium has the following formula: Part a: Sodium chloride 8 g, nutrient agar 1.5% (Difco) 4 g, distilled water, to make 900 cc; Part b: Human plasma (citrated) 100 cc, human hemoglobin 20 cc.‡

The base (part a) is sterilized in the autoclave, and part b added later. The medium is dispensed in test tubes and corked with rubber stoppers. The final pH is 7.4-7.5. It will be seen that the medium is a modification of that used by Noguchi and Battistini[2] for the cultivation of *Bartonella bacilliformis*.

Cultures are incubated at room temperature (26-28°C), and become positive 7 to 10 days after inoculation, even when the original inoculum contains no microscopically demonstrable trypanosomes, but the tubes should not be discarded until after one mon'h. The cultures remain viable for 60 days or more, the maximum thus far observed being 71 days. After 2 weeks of cultivation, the pH of the medium drops to 7.2.

Thus far isolations from inoculated susceptible animals have been regularly successful.

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1 Brutsaert, P., and Henrard, C., *C. R. Soc. de Biol.*, 1938, **127**, 1469.

† Obtained through the kindness of Dr. Henry D. Murray and Dr. G. G. Campbell.

‡ Made by laking 1 part of blood with 3 parts of distilled water.

2 Noguchi, H., and Battistini, T., *J. Exp. Med.*, 1926, **43**, 851.