Results. By the third week of treatment six of the nine animals remaining were hypertensive, with blood pressures ranging from 156 to 206 mm Hg, one having died prior to this. By the 27th day two others had died and all of the surviving methyl cellulose-treated rats were hypertensive, with pressures ranging 168–234 mm Hg. None of the controls became hypertensive. In the last week of treatment four of the injected rats died with severe ascites, edema, pleural effusion, or a combination of these. A typical example of severe edema is shown in the Figure. At autopsy the hearts were noted to be enlarged and showed small surface scars. The kidneys were yellowish, usually moderately enlarged, and had an irregular surface.

Hypertensive rats had marked cardiac hypertrophy, their hearts being significantly heavier than controls ($P < 0.001$), due chiefly to thickening of the left ventricular wall. The adrenal glands and spleens were not enlarged, being if anything slightly reduced in size. The data on blood pressure and organ weights are given in the Table. Vascular lesions of the type associated with hypertension observed in various organs, and 'foam cell' transformation of glomerular endothelial elements, were both evident on microscopic examination. They are under current study and will be the subject of a later communication.

Severe edema in a rat which had received methyl cellulose for 32 days. Weight 212 g, blood pressure 210 mm Hg. The animal also had marked ascites.

Principal findings in methylcellulose-treated and control rats

Data			Methylcellulose-treated	Controls
Body weight g	Initial		83 ± 2[a]	83 ± 2
	Final		179 ± 11	183 ± 4
Blood pressure	Day 21		157 ± 14	120 ± 3
mm Hg	27		197 ± 12	121 ± 2
	32		200 ± 10	124 ± 2
Organ weights	Adrenals	mg	48.1 ± 3.0[b]	62.1 ± 2.4
	Spleen	mg	467 ± 74[b]	602 ± 24
	Heart	mg	788 ± 29[b]	585 ± 19
	Kidney	g	2.00 ± 0.12[b]	1.88 ± 0.21

[a] Mean ± S. E. of mean.
[b] Based on 3 animals killed and 4 which died in the same week.

Discussion. The physical manifestations of methyl cellulosis include edema, ascites and pleural effusions and, as this experiment shows, arterial hypertension, cardiac enlargement and vascular lesions. The concomitant morphologic transformation of the glomeruli into enlarged 'foam cell' laden structures makes it seem likely that the cardiovascular effects are the sequelae of reduced intrarenal circulation. In contradistinction to other methods of interfering with kidney blood flow and thereby causing hypertension (such as partial occlusion of the renal arteries or the investment of kidneys in a semi-rigid capsule) the present method appears to depend, at least initially, upon impairment of circulation at the glomerular level.

The appearance of the transformed glomeruli as published by others[3,4] and observed by us, is somewhat reminiscent of that sometimes seen in lipoid nephrosis[5] and certain other forms of glomerulonephrosis, excepting that in methyl cellulose thesaurosis the endothelial cells contain the macromolecular polysaccharide instead of lipid. The occurrence of hypertension and edema both in the human and experimental conditions would suggest the possibility that the physiological aberrations in each are evoked by a closely related if not identical mechanism. This would seem to be dependant upon both reduced intrarenal circulation and direct impairment of glomerular filtration. Although in the present study the animals were sensitized to hypertension by uninephrectomy and the imposition of a high NaCl intake, recent experiments in this laboratory indicate that neither is essential to the production of experimental hypertension by this means. Further studies are in progress to elucidate the nature of the mechanism which leads to the development of hypertension.

Résumé. Des injections sous-cutanés d'une solution de méthyl-cellulose provoquent dans la rate une vraie hypertension artérielle maligne. Les animaux utilisés eurent fréquemment des œdèmes et des ascites et toujours des lésions artérielles dans le cœur, les reins et ailleurs.

C. E. Hall and O. Hall

The Carter Physiology Laboratory, University of Texas Medical School, Galveston (Texas), August 3, 1961.

Isolation of 5-Hydroxytryptamine from the Skin of the Toad *Bufo arenarum* Hensel

B. arenarum Hensel is the widest spread species of toad in Argentina and has been in common use in this country for physiological and pharmacological work.

From the dried paratoid secretion, usually called venom, bufotenine and dehydrobufotenine[1]; adrenaline[2], bufothionine[3], and from the dried skins, bufothionine, bufotenine and dehydrobufotenine[4a] were obtained. The secretion also contains several bufogenines, which have been recently identified[5]. We now record the isolation of 5-hydroxytryptamine from the dried skins of *B. arenarum*, a compound which has been detected in several species of toad[6].

1 K. K. Chen, H. Jensen, and A. L. Chen, J. Pharmacol. exp. Therap. *49*, 1 (1933).
2 V. Deulofeu, Hoppe Seylers Z. physiol. Chem. *237*, 171 (1935).
3 H. Jensen, J. Amer. chem. Soc. *57*, 1765 (1935).
4 (a) H. Wieland, W. Konz, and H. Mittasch, Liebigs Ann. Chem. *513*, 1 (1934). – (b) V. Deulofeu and E. Duprat, J. biol. Chem. *153*, 450 (1944).
5 R. Rees, O. Schindler, V. Deulofeu, and T. Reichstein, Helv. chim. Acta *42*, 2400 (1959), where the former papers on the genins of *B. arenarum* Hensel are mentioned.
6 V. Erspamer, Pharmacol. Rev. *6*, 425 (1954).

Extracts from dried skins (149 skins weighing 430 g) were fractionated as described by DEULOFEU and DUPRAT[4b]. After extraction of the bufotenine, the barium ions were precipitated as sulfate, the remaining solution brought to pH 6 and concentrated to 50 ml. This concentrate on paper chromatography[7], gave spots corresponding to bufotenine, dehydrobufotenine and 5-hydroxytryptamine. It was then saturated with ammonium sulfate and extracted with *n*-butanol. The 5-hydroxytryptamine in the butanol phase was precipitated by addition of 5-nitrobarbituric acid, and crystalline 5-hydroxytryptamine was prepared following the usual procedure[8]. Material recrystallized from acetone-water had m.p. 207–210° (Kofler), undepressed when mixed with an authentic sample, Rf 0.12 (*n*-butanol saturated with *N* hydrochloric acid) and 0.43 (*n*-butanol : acetic acid : water; 4:1:5).

The presence of 5-hydroxytryptamine was also detected by paper chromatography in a sample of the dried secretion of the paratoid glands of the same species of toad, kindly given to us by Prof. J. A. IZQUIERDO.

Zusammenfassung. 5-Hydroxytryptamin wurde aus der Haut der in Argentinien häufigsten Krötenart *B. arenarum* Hensel isoliert und in trockenen Paratoidsekreten chromatographisch nachgewiesen.

B. FRYDMAN and V. DEULOFEU

Laboratorios de Investigación, E. R. Squibb & Sons Argentina S.A., Martínez (Prov. Buenos Aires, Argentina), August 24, 1961.

[7] V. ERSPAMER and G. BORETTI, Arch. int. Pharmac. Therap. *88*, 313 (1951).
[8] M. M. RAPPORT, A. A. GREEN, and I. H. PAGE, J. biol. Chem. *174*, 737 (1948); Science *108*, 329 (1948).

The Uptake of Pyrithiamine by Cerebral Tissue

The only *in vitro* effect of pyrithiamine (PT) is the inhibition of thiamine (T) phosphorylation[1–4] by thiaminokinase, which is considered to explain the antivitamin mechanism of PT[5].

In vivo a particular action of PT was shown by DE CARO et al. in rats[6–8] and mice[9,10]. The oral or intraperitoneal administration of the antivitamin, at doses ranging from 0.2 to 6 mg, alone or together with small amounts of T, as a single dose or repeated for 4–6 days, always caused an earlier and deeper decrease of T (total and cocarboxylase) content in brain than in other organs.

This must be considered a distinctive feature of PT athiaminosis as compared with nutritional athiaminosis, where the brain is known to preserve its T content for a longer time than other organs[11–13].

As a consequence of this action of PT on cerebral tissue, most animals show, in 10–15 days, the typical neurological signs of beri-beri, which are difficult to produce in rodents, even in extreme nutritional T deficiency. These results have been confirmed by KOEDAM in pigeons[5] and mice[14]. It is clear that knowledge of the PT tissue levels after PT administration could clarify its apparent specificity in lowering the total T (esterified T) content of the brain. In fact, the hypothesis can be made that PT accumulates preferably in the cerebral tissue, displacing T and thus causing the early onset of the beri-beri symptoms.

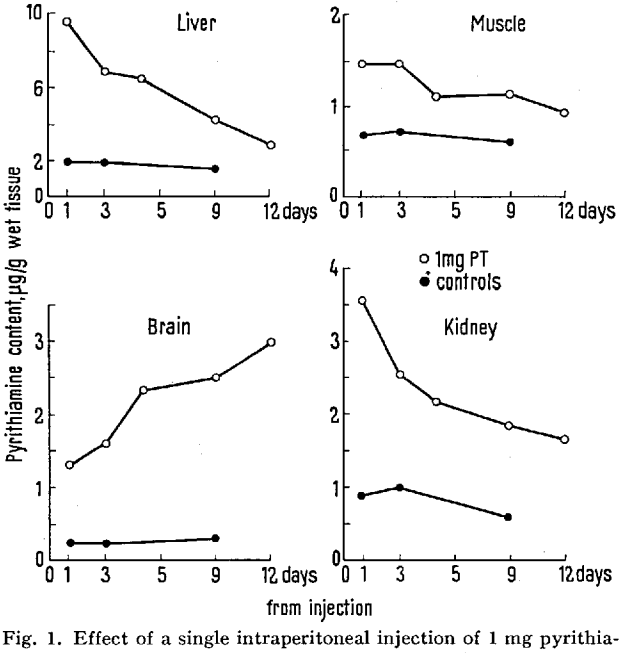

Fig. 1. Effect of a single intraperitoneal injection of 1 mg pyrithiamine (PT) on the PT-content in various organs. Rats reared on a thiamine-deficient diet. Each point is the average of 7 determinations.

The specific microfluorimetric method of RINDI and PERRI[15,16] for the determination of PT in presence of T enabled us to verify this hypothesis. The experimental conditions chosen were quite similar to those in which we found the earliest and the most striking decrease of the cerebral T content[8]. A single dose of 1 mg of PT (Calif. Biochem. Corp., Los Angeles), dissolved in 0.2 ml of saline, was injected intraperitoneally in male albino rats (body weight 80–90 g). The animals, reared on standard T-deficient diet[8], were sacrificed at different time intervals after injection. In brain, liver, muscle, and kidney of treated and control (untreated) rats the PT content was determined. The results are summarized in Figure 1. As is shown, the PT content found in all organs except brain 24 h after injection, was approximately that of T usually present. Successively, the PT content decreased in liver,

[1] D. W. WOOLLEY, J. biol. Chem. *194*, 45 (1951).
[2] S. EICH and L. R. CERECEDO, J. biol. Chem. *207*, 295 (1954).
[3] L. R. CERECEDO, S. EICH, and E. BRESNICK, Biochim. biophys. Acta *15*, 144 (1954).
[4] J. C. KOEDAM and E. P. STEYN-PARVÉ, Konikl. Nederl. Akad. Wetenschap. Proc. Ser. C *63*, 318 (1960).
[5] J. C. KOEDAM, Biochim. biophys. Acta *29*, 333 (1958).
[6] L. DE CARO, G. RINDI, and E. GRANA, Exper. *10*, 110 (1954).
[7] L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI, Int. Vitaminforsch. *26*, 345 (1956).
[8] L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI, Int. Vitaminforsch. *28*, 253 (1958).
[9] L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI, Exper. *12*, 300 (1956).
[10] L. DE CARO, G. RINDI, and G. FERRARI, Int. Z. Vitaminforsch. *29*, 40 (1958).
[11] J. SALCEDO jr., V. A. NAJJAR, L. E. HOLT jr., and E. W. HUTZLER, J. Nutr. *36*, 307 (1948).
[12] G. RINDI, G. FERRARI, and V. PERRI, Int. Z. Vitaminforsch. *25*, 210 (1954).
[13] H. P. G. WESTENBRINK, Arch. Neerl. *17*, 560 (1932).
[14] J. C. KOEDAM and E. P. STEYN-PARVÉ, Konikl. Nederl. Akad. Wetenschap. Proc. Ser. C *62*; 101 (1959).
[15] G. RINDI and V. PERRI, Boll. Soc. ital. Biol. sper. *35*, 2025 (1959).
[16] G. RINDI and V. PERRI, Biochem. J. *80*, 214 (1961).