fate. It is therefore concluded that the new bone on the host side of the filter must have come from the host. With regard to the possibility that the Millipore filters per se were responsible for the bone induction, this seems unlikely in view of my inability in other experiments to demonstrate bone formation around freely implanted Millipore filter material as well as in view of the failure of other investigators (*4*) to note new bone formation on the host side of Millipore filters. Rather, the experimental results indicate that the new, vital bone found in immunized mice on the host side of diffusion chambers containing homograft bone is derived from host tissue in response to a *diffusible osteogenic inductor* coming from the new bone laid down on the inner aspect of the filter, thereby representing the in vivo extension of the in vitro findings of Grobstein (*5*) and Lash *et al.* (*6*) with respect to the passage of inductor substances through Millipore filters (*7*).

PAUL GOLDHABER
Harvard School of Dental Medicine, Boston, Massachusetts

References and Notes

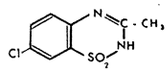
1. P. Goldhaber, *Proc. Soc. Exptl. Biol. Med.* **98**, 53 (1958).
2. G. H. Algire, J. M. Weaver, R. T. Prehn, *J. Natl. Cancer Inst.* **15**, 493 (1954).
3. ———, *Ann. N.Y. Acad. Sci.* **64**, 1009 (1957).
4. R. B. Duthie, *Brit. J. Plastic Surg.* **11**, 1 (1958); L. A. Hurley, F. E. Stinchfield, C. A. L. Bassett, W. H. Lyon, *J. Bone and Joint Surg.* **41A**, 1243 (1959).
5. C. Grobstein, in *Aspects of Synthesis and Order in Growth*, D. Rudnick, Ed. (Princeton Univ. Press, Princeton, N.J., 1955), p. 233.
6. J. Lash, S. Holtzer, R. Holtzer, *Exptl. Cell Research* **15**, 292 (1957).
7. Further experiments are now in progress to determine the specificity and chemical nature of the diffusible osteogenic inductor reported here. This study was supported by the U.S. Army, Office of the Surgeon General, under contract No. MD-2018. I gratefully acknowledge the technical assistance of Miss G. Cirulis and G. Pettengill.

20 February 1961

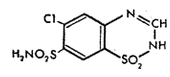
New Class of Antihypertensive Agents

Abstract. Effective antihypertensive agents of the benzothiadiazine series, devoid of diuretic activity, are described. There follows a method of synthesis and a description of the pharmacological activity of one of these substances.

We have synthesized 7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide (I), the first compound in the 1,2,4-benzothiadiazine-1,1-dioxide series in which a separation of the antihypertensive and diuretic activities has been achieved. A characteristic of the diuretic agent chlorothiazide (*1*) (II) and other related benzothiadiazine diuretics, including the 3,4-dihydro derivatives, is their property of moderately reducing the blood pressure of hypertensive subjects.



I



II

Although the precise mechanism of this action is not known, it has generally been assumed to be related to the diuretic and natriuretic properties of the compounds (*2*).

Compound I was synthesized as follows: 2,4-dichloronitrobenzene was converted into 2-benzylthio-4-chloronitrobenzene, with a melting point of 132° to 134°C (found: N, 5.03) by reaction with benzylmercaptan in the presence of potassium hydroxide in ethanol solution. Treatment of the benzylthio compound with chlorine in aqueous acetic acid, followed by reaction of the resulting sulfonyl chloride with ammonia, afforded 5-chloro-2-nitrobenzenesulfonamide, with a melting point of 159° to 160°C (found: N, 11.71; Cl, 14.53). Reduction of the latter with iron filings and ammonium chloride solution yielded 2-amino-5-chloro-benzenesulfonamide, with a melting point of 152° to 153°C, λ_{max} (MeOH), 253 mμ (ϵ, 12600), 321 mμ (ϵ, 3100); λ_{max} (Nujol), 6.14 μ (found: N, 13.27; Cl, 17.22) which, upon heating with ethyl orthoacetate at 100° to 110°C, furnished 7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide (I), with a melting point of 330° to 331°C, λ_{max} (MeOH), 268 mμ (ϵ, 11300), λ_{max} (Nujol), 6.22 μ (found: N, 12.40; Cl, 15.41).

When compound I was administered orally at a dose of 5 mg/kg per day (in two divided doses) to renal hypertensive dogs, a gradual (in 2 to 6 days) fall in blood pressure was observed, which was maintained for the duration of the experiment (12 days) without evidence of diuresis. Upon withdrawal of the drug, the blood pressure returned to approximately pretreatment levels in 3 to 6 days. Essentially similar antihypertensive effects were obtained by using metacorticoid hypertensive rats. These results have also been confirmed clinically, the extent of the pressure reduction in many cases exceeding that observed with the benzothiadiazine diuretics.

Chemically, compound I differs importantly from the diuretic 1,2,4-benzothiadiazine-1,1-dioxides in being devoid of the benzenoid sulfamyl group. Further experiments have indicated that other compounds of this type and other classes of compounds which differ from known diuretic sulfonamides in that the sulfamyl group is replaced by hydrogen, alkyl, halogen, trifluoromethyl, or the like, demonstrate a similar antihypertensive activity separate from diuretic action. It thus appears, from our studies, that removal of the sulfamyl group from substances having diuretic properties usually results in compounds without diuretic effect but exhibiting antihypertensive activity. We are synthesizing and biologically evaluating an extensive series of these compounds.

ALAN A. RUBIN, FRANKLIN E. ROTH, MARTIN M. WINBURY, JOHN G. TOPLISS, MARGARET H. SHERLOCK, NATHAN SPERBER, JACK BLACK
Schering Corporation, Bloomfield, New Jersey

References

F. C. Novello and J. M. Sprague, *J. Am. Chem. Soc.* **79**, 2028 (1957).
F. A. Tapia, H. P. Dustan, R. E. Schneckloth, A. C. Corcoran, I. H. Page, *Lancet* **2**, 831 (195
E. D. Freis, A. Wanko, I. M. Wilson, A. E. Parrish, *Ann. N.Y. Acad. Sci.* **7**, 450 (1958).

5 May 1961

Stereotypy and Intermittent Reinforcement

Abstract. Three pigeons were trained to peck at a horizontally oriented rubber srtip 10 in. long. The spatial distribution of responding along this strip is found to be nonrandom when every peck is reinforced with food. The degree of nonrandomness increases markedly when the pecking is intermittently reinforced.

Antonitis demonstrated that hungry rats will form strong preferences for particular locations when they are given equal opportunities to be fed at a number of different locations (*1*). The experimental situation he used consisted of a box that had a long, narrow, horizontal slot in one wall, and the rat was given a single pellet of food after it put its snout into any part of the slot. Each rat developed a marked preference for some location along the slot. Later, Antonitis discontinued de-