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*).

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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.

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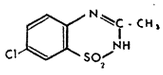
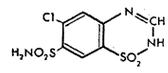
New Class of Antihypertensive Agents

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.

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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-

livering pellets for this behavior and found that as the behavior disappeared it went through a phase of much-increased variability. Other experimenters (*2*) have also noted an increase in the variability of a learned response during the course of its extinction, and some have commented on the adaptiveness of this increase in bringing out new forms of behavior.

Antonitis reported that the stereotypy of responding returned when the behavior was again reinforced with pellets. The stereotypy after reconditioning was even greater than the stereotypy during the original training. These findings of Antonitis raise an interesting question about the relation between variability of responding and intermittency of reinforcement. The increase in variability during extinction suggests that a schedule of reinforcement that does not reinforce every occurrence of the selected response will generate more variability than a schedule that does. The heightened stereotypy in reconditioning seems to imply the opposite, namely that a schedule in which the animal is intermittently reinforced (that is, reconditioned) will generate less variability.

The experiment reported here (*3*) was an attempt to establish merely whether variability increases or decreases as a result of intermittent reinforcement. Pigeons were used as subjects in an experimental chamber that contained a 10-in. by 1-in. rubber strip horizontally centered on one wall. A feeding device could be activated to give the pigeon access to grain for about 3 sec. The pigeons were trained to peck at the rubber strip, and the location of the peck was automatically recorded within one of ten 1-in. squares. Three pigeons were used.

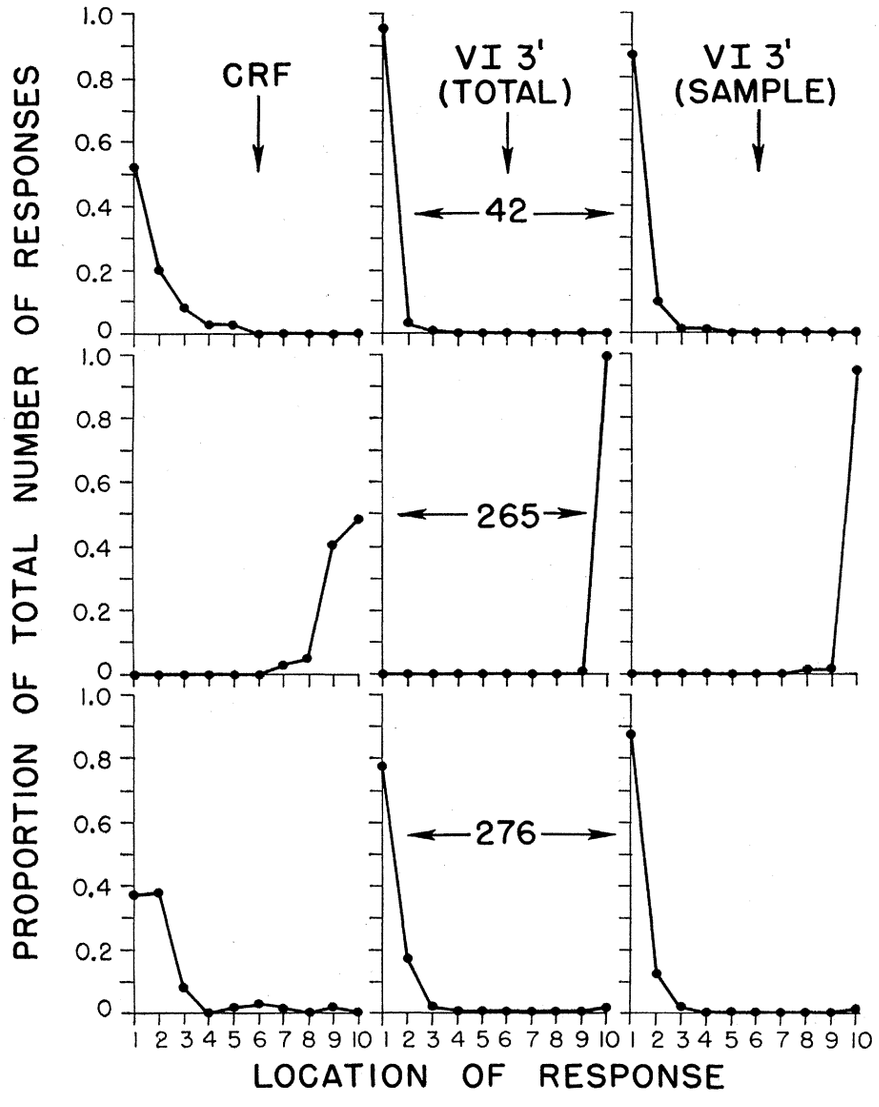

Fig. 1. Relative frequency of responding is shown as a function of location on the response-strip. Each horizontal row of graphs is for one pigeon. The first column of graphs is for a schedule of continuous reinforcement; the second and third columns are for intermittent reinforcement.

In the first phase of the experiment, any peck on the rubber strip operated the feeding device. Approximately 2 wk of daily experimental sessions were conducted; each session consisting of 60 reinforcements. In the second phase, the pigeons were reinforced according to a variable-interval schedule. Pecking the rubber strip operated the feeding device every 3 min, on the average. The range of time intervals between reinforcements in this schedule was from about 5 sec to about 7 min. Reinforcements were still given without respect to the location of the peck on the rubber strip. Sessions were again conducted until the pigeon received 60 reinforcements.

Figure 1 summarizes the result of the experiments. Each horizontal row of graphs is for one pigeon; each vertical column for a particular measure of behavior. The points on the graphs are medians from five sessions. In each graph, frequency of responding, expressed as a proportion of the total frequency of responding, is shown as a function of the location on the rubber strip. The ten locations are numbered 1 to 10, going from left to right. The area under each graph approximates 1.0. The first column of graphs, labeled "CRF," (that is, continuous reinforcement) shows the distribution of responding obtained when each peck to the strip was reinforced. A horizontal line at 0.1 would indicate a random distribution of responding, and these graphs make it clear that some degree of stereotypy is present. Each pigeon manifests a tendency to peck at one or the other end of the rubber strip. The second column of graphs shows that the intermittent schedule of reinforcement ("VI 3′" or variable interval of 3 min) produced increased stereotypy. With the first procedure the modal location got between 40 and 52 percent of the responding, with the second procedure the range was from 78 to 99 percent. This increase in stereotypy was evident in the very first experimental session with intermittent reinforcement. The continuous records of location of responding showed that there was increased responding on the modal loca-

tion after about 15 min of the first session and that the final level was reached by the second or third session.

The third column of graphs is a check on possible artifacts in the results. Under the first procedure, the pigeon moved away from the rubber strip after every peck in order to go to the feeding device. Under the second procedure, only a small fraction of the pecks were followed by a movement to the feeding device. It seemed possible that the return to the rubber strip after eating caused a certain amount of variability in the location of responding. This factor would dominate the first procedure, but would be only negligible in the second. The third column of graphs is therefore based on only those pecks, under the variable-interval schedule, that immediately followed eating. Since there were 60 reinforcements in each session, this sample included 59 pecks per session. The distributions of responding in the third column show as much stereotypy as those in the second. It is therefore evident that the increase in stereotypy produced by intermittent reinforcement is due neither to the animal's being forced to move around in the situation nor to the large difference between the numbers of responses that entered into the computation of the first and second columns of graphs.

The duration of sessions under continuous reinforcement was about 10 min; under variable-interval reinforcement it was about 3 hr. This difference in time may have been responsible for the difference in variability under the two procedures. The distributions of responding during the first 10 min of sessions under variable-interval reinforcement make this explanation implausible. The amount of variability seen here was greater than that shown in the second and third columns of Fig. 1, but was still well below the amount shown in the first column.

This experiment strongly suggests that stereotypy is enhanced by intermittency of reinforcement itself and not by some essentially trivial concomitant of the change in procedure from continuous to intermittent reinforcement. Stereotypy is to be expected when there is no benefit to the animal for moving around in the situation. The principle of reinforcement predicts that a location that is reinforced early in the animal's exposure to the procedure would gradually come to dominate the distribution of responding. Moreover, the spread of responding in extinction is empirically, although not theoretically, expected. Why responding becomes more stereotyped during intermittent reinforcement does not, however, follow easily from our present state of knowledge. The clarity of the present findings indicates that a significant but unexplored principle may be involved.

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27 February 1961

A Bacteriophage Specific for F— Salmonella Strains

Abstract. A bacteriophage is described which grows on recipient (F—) and not on donor (Hfr, F+, or F′) salmonella. The reason for this differential is unknown, since the phage attaches to all mating types, but progeny ensue only from female bacteria. Purification of this phage has been accomplished. A procedure is described for quantitatively determining either the acquisition or loss of the F agent by bacteria.

Loeb (*1*) and Loeb and Zinder (*2*) reported on a bacteriophage, f2, which would differentiate donor (F+ or Hfr) from recipient (F—) *Escherichia coli* K-12. The phage grows only on donor bacteria. Zinder (*3*) described a staining reaction involving the interaction of *Salmonella typhimurium* strains and *Escherichia coli* strains which could be used to differentiate donor and recipient *S. typhimurium* and *E. coli*. The present report describes a bacteriophage which grow only on recipient *S. typhimurium* strains and not on donor strains. Hereafter, donor strains are referred to as males and recipients as females.

The bacteriophage SP6 was isolated from sewage some years ago by Lederberg (*4*) as one that would grow on *S. typhimurium*, and it was in the collection of salmonella phages listed by Zinder (*5*). In the course of developing some bacterial mutants that were resistant to SP6 for use as a genetic marker in studies (*3*) of recombination in *S. typhimurium* LT2, it was noted that the phage failed to grow on the male bacteria. Since LT2 is female (*3*), as are all but certain of its derivatives, it was possible to compare the growth of SP6 on a whole series of male cultures for which the immediate female ancestor was available. With ten such paired cultures, SP6 grew with equal efficiency on the females but with an efficiency of less than 10^{-6} on males. The male set included eight F+ (*6, 7*) strains, one F′ strain (*8*), and one Hfr strain (*6*). The techniques for isolating such salmonella strains were described by Zinder (*3*). It was noted that when the phage was plated at high concentration on F+ and F′ strains (but not when it was plated on Hfr strains) there was some clearing in the background of the plates but in an amount insufficient to form discrete plaques. This is because F′ and F+ strains both contain significant numbers of F— "mutants." The technique for demonstrating this will be described later.

To verify further this correlation of phage sensitivity and mating type, the inheritance of sensitivity to SP6 was studied. The criterion used to determine mating type of the progeny of a cross was based on the staining reaction. Two crosses were done with the same pairs of selective markers, except that in one the male was an F+ and in the other an Hfr which had been derived from it. In *Salmonella typhimurium* as in *Escherichia coli,* the progeny of an F+ by an F— cross are generally F+, while progeny from Hfr by F— crosses are F—. The crosses were histidine-requiring males by a leucine-requiring female. Progeny were selected on the basis of nutritional independence. The progeny were isolated, purified, and then tested for the staining reaction and phage sensitivity. There was a perfect correlation in the F status of the progeny as determined by these two criteria. Ninety-five percent of the progeny from the F+ cross were F+, and only 2 percent of the progeny from the Hfr cross had the F agent.

Another test of the correlation of phage sensitivity with mating type could be based on the fact that with *E. coli* there is efficient transfer of F by the simple growth of F+ and F— organisms in mixed culture (*6, 7*). This does not occur with Hfr cultures. With