whereas sulfathiazole in a dose equimolar to the absolute lethal dose of its sodium salt killed 50% of the animals. The toxic picture caused by sulfathiazole was not unlike that seen after injection of its sodium salt.

The results of van Dyke and associates with oral administration[3] are similar to our findings. They report sodium sulfathiazole to have about 65% of the toxicity of sodium sulfapyridine, whereas upon feeding large amounts of the free compounds, sulfathiazole appeared to be the more toxic.

It is obvious from the data presented that toxicity determinations with the sodium salts of sulfanilamide derivatives having different alkalinities will not give a true picture of the toxicity of the free compounds. At present, therefore, the free compounds injected intraperitoneally as suspensions are being compared.

Conclusions. It has been shown that the acute toxicity of sodium sulfamethylthiazole in rats, as measured by intraperitoneal injection, is almost twice as high as that of sodium sulfathiazole, which in turn is slightly greater than the toxicity of sodium sulfapyridine.

The pH and buffer capacities of solutions of the sodium salts, and differences in solubility of the free compounds as factors influencing the toxicity, are discussed.

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Retention of Water by Pituitary (Posterior Lobe) Extract in Winter Frogs.

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The present report deals with further data bearing upon the mechanism of seasonal variation in the Brunn Reaction. The Brunn Reaction consists of an uptake of water by frogs when injected with suitable doses of pituitary (posterior lobe) extract and it is quantitatively greater in the summer than in the winter.[1] Certain aspects of the seasonal variation have been investigated in this laboratory. The reported increase in the winter of the number of acidophilic cells in the buccal lobe of the frog hypophysis lead us to inject various anterior pituitary and anterior pituitary-like principles along with extract of the posterior lobe but these additions did not depress the

[1] Boyd, E. M., Mack, E. G., and Smith, A. E., *Am. J. Physiol.*, 1939, **127**, 328.

Brunn Reaction in summer frogs.[2] A corresponding seasonal variation does not occur in albino rats given water by mouth with and without posthypophyseal extract.[3]

Boyd and Whyte[4] presented evidence which suggested that the Brunn Reaction is due not to a stimulation of water intake, which is the explanation usually offered, but to an inhibition of the normal loss of water by frogs immersed in water and to a consequent retention of water which is taken up over the time interval of the Brunn Reaction. The experiments which lead to this hypothesis consisted of measurement of the effect of pituitary (posterior lobe) extract on the loss of normal body water and of water injected into frogs which are taken out of water at the beginning of the period of measurement, in contrast to the procedure in the Brunn Reaction in which they are left in water throughout. Under these conditions, doses of pituitary (posterior lobe) extract corresponding to those used in the Brunn Reaction caused a retention of normal body water and of water injected into frogs. Further experiments demonstrated that dehydrated frogs are capable of taking up, and 'hydrated' frogs are capable of losing per hour amounts of water corresponding to those taken up and retained in the Brunn Reaction. Correlating these observations, the above explanation of the Brunn Reaction was offered.

The experiments of Boyd and Whyte[4] were performed in the summer months of 1938 and were confirmed in this laboratory by Mr. J. E. Brown in the summer of 1939. In January of 1940, several separate extended studies on what we may call the Boyd-Whyte Reaction were undertaken. None of us were able to obtain a normal Boyd-Whyte Reaction in spite of many repetitions. It occurred to us that this phenomenon might be due to a seasonal variation since experiments on the Boyd-Whyte Reaction had not been previously made during the winter in this laboratory. Repeating our experiments each week, we found that the Boyd-Whyte Reaction became established again rather suddenly toward the end of April and first of May at about the same time that the Brunn Reaction reaches its summer proportions.[1] We wish to report these changes in the Boyd-Whyte Reaction which accompany seasonal variation in the Brunn Reaction.

Method. Leopard frogs *(R. pipiens)* were acclimatized to room temperature in water for a period of 18 hours or longer in the months

[2] Boyd, E. M., and Gibson, J. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 572.

[3] Boyd, E. M., and Garand, N. D., *Am. J. Physiol.*, 1940, **130**, 403.

[4] Boyd, E. M., and Whyte, D. W., *Am. J. Physiol.*, 1939, **125**, 415.

of January, February and March of 1940. At the beginning of a period of measurement, they were removed from water, urine expressed the skin thoroughly dried and body weight determined to the nearest 0.1 g on a trip scale balance. Half of the animals in each group received into the dorsal lymph sac an injection of a given dose of Pituitrin Surgical (generously supplied by Dr. E. A. Sharp of Parke, Davis and Company which company also provided a grant that defrayed part of the expenses of this investigation). The remaining half of the animals in each group served as controls and received by injection an equivalent volume of the vehicle of Pituitrin Surgical. Both groups were then re-weighed at hourly intervals for 5 to 6 hours. This procedure was repeated until there was a total of at least 50 frogs to a group. Percentage changes in body weight were then calculated and plotted against time.

Normal Body Water. When frogs were taken out of water at room temperature in the winter months they were found to lose water at the rate of approximately 1% per hour. When pituitary (posterior lobe) extract was injected into these frogs in doses of 0.1 to 0.5 international units per 10 g body weight (corresponding to doses used in the Boyd-Whyte Reaction), the animals continued to lose weight at exactly the same rate. In other words, the Boyd-Whyte Reaction could not be demonstrated at this season of the year. At the same season of the year, the Brunn Reaction had decreased to about one-half its summer maximum but did not entirely disappear as did the Boyd-Whyte Reaction.

Dosage of Extract. It was considered possible that frogs in the winter months had merely lost their sensitivity to pituitary (posterior lobe) extract and the above experiments were repeated using a wide range of doses from 10^{-15} to 20 international units per 10 g of frog. For doses above 5 units, we could not use Pituitrin Surgical because the presence of chlorbutol in the vehicle rendered the extract lethal to frogs in such large amounts. We accordingly prepared our own extract from standard pituitary powder containing 2 international units per milligram by the method outlined in the British Pharmacopoeia, 1932, and this extract was used in the experiments with high doses. All of the results were entirely negative, even with the high doses. We had to conclude that the loss of normal body water of frogs could not be prevented in winter by doses of pituitary (posterior lobe) extract up to 20 international units per 10 g of frog (equivalent to 15 liters of Pituitrin Obstetrical injected into an average woman).

Administered Water. Boyd and Whyte[4] also demonstrated that when frogs in the summer are injected with 10% of their weight of

distilled water into the dorsal lymph sac, a simultaneous intramuscular injection of Pituitrin Surgical in doses of 0.1 to 0.5 international units per 10 g will cause retention of the injected water as well as of normal body water. Repeating this experiment in the winter, we were able to show that pituitary (posterior lobe) extract is still capable of causing a retention of some of the injected water although it has lost the power to cause a retention of normal body water. In experiments on some 300 frogs, the mean retention of injected water was 40% over a period of 3 hours. In over 90% of the frogs the injected water was retained by the neurohypophyseal extract. The percentage retention of injected water varied considerably but on the average was below that in the summer, at which time up to 100% retention was recorded in the first 3 hours.

These results demonstrate that pituitary (posterior lobe) extract has an effect on administered water different from that on normal body water because at a time when the extract was not capable of causing a retention of normal body water it was capable of causing a retention of administered water. The results of the present study of the Boyd-Whyte Reaction and those of Boyd, Mack and Smith[1] on the Brunn Reaction combine to demonstrate a truly remarkable seasonal variation in the water metabolism of frogs.

Conclusion. The depression of the Brunn Reaction in winter months reported by Boyd, Mack and Smith[1] was found accompanied by complete failure of pituitary (posterior lobe) extract to inhibit loss of normal body water in frogs. On the other hand, the loss of water injected into frogs was inhibited by pituitary (posterior lobe) extract in the winter though to an average extent less than in the summer.

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Modifications in Development of the Sand Dollar by NaCNS.

OLIN RULON. (Introduced by C. M. Child.)

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Experiments conducted at the Hopkins Marine Station, Pacific Grove, California, during the summer of 1938 on the unfertilized and fertilized eggs of *Dendraster excentricus* have given certain interesting modifications in the early developmental patterns of this form.