shift. A frequency of 9 c/s corresponds to 55 msec lag. Adding an additional lag of 100 msec would reduce the frequency of oscillation by a factor of nearly three; but the shift in our experiments was always much less than this. Hence the 9 c/s peak is not of this nature. Suppose instead that the 9 c/s peak is a harmonic of some fundamental mode of oscillation at a lower frequency. It turns out that if it were the third harmonic, the fundamental being at about 3 c/s (corresponding to an inherent lag of 167 msec), the expected shift in the 9 c/s peak with added delay would be reconciled with the experimental results, and, furthermore, the two higher frequency peaks, when they occur, would fall into place as the fifth and seventh harmonics. This identification has therefore much to recommend it, but, from other considerations, we prefer a different, but closely related, working hypothesis in which the fundamental (if present) corresponds to an oscillatory tendency with π radians of phase shift round the loop, but the higher peaks are not harmonics but separate modes of oscillation with 3π , 5π and 7π radians phase shift. We prefer this hypothesis because it can account (a) for the apparent lack (in most experiments) of a peak at 3 c/s (any mode may be absent if the gain at that frequency is low), (b) for the absence of peaks corresponding to the even harmonics (instability only results when the phase shift is $\pi + 2n\pi$ radians) and (c) for the occurrence of multiple peaks not in any simple harmonic relation when frequency dependent lags are introduced. These last observations will be described in the full account of this work now in preparation.

It should be mentioned that not all 9 c/s peaks are visually determined. By slightly altering the mechanical conditions of the task Dymott and Merton⁴ have produced good 9 c/s peaks in runs with the eyes shut.

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Received September 21, 1967.

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Dissociation of the Pressor and Antidiuretic Activities of Synthetic Arginine Vasopressin by Heating at pH 10

Zaoral, Koleč and Šorm¹ showed that the analogue of arginine vasopressin in which the arginine is in the D rather than the L-configuration, [8-D-arginine]-vasopressin, has ten times as much antidiuretic as vasopressor activity. This finding allows an explanation for the early report by Heller² that a similar dissociation of activities was obtained when 'Pitressin' (Parke, Davis and Co.) was heated at alkaline pH. One of the effects of such treatment on polypeptides is racemization of their constituent amino-acids, and, if one can generalize from the behaviour of adrenocorticotrophin (ACTH), one of the amino-acids most likely to racemize is arginine³.

Fraser⁴ confirmed Heller's² results when he assayed antidiuretic activity by subcutaneous injection of the hormone into rats or dogs. When, however, he administered the hormone intravenously, to dogs he found that the antidiuretic potency of the treated hormone, so determined, was the same as its vasopressor potency. He attributed the discrepancy between the antidiuretic potencies, determined by the subcutaneous and intraven-

Table 1. EFFECT OF HEATING SYNTHETIC ARGININE VASOPRESSIN AT pH 10 IN A BOILING WATER BATH ON THE RATIO OF ITS ANTIDIURETIC AND VASOPRESSOR ACTIVITIES

Time of heating (min)		Potency (U/ml.)*		Antidiuretic Vasopressor
		Antidiuretic	Vasopressor	
Unheated	(a)	2.147 (1.863-2.438)	1.944 (1.572-2.393)	1.06
	(b)	2.510 (2.241-2.846)	2.172 (2.041-2.314)	1.15
15	(a)	0.468 (0.410-0.526)	0.371 (0.330-0.422)	1.26
30	(b)	0.117 (0.092-0.156)	0.038 (0.034-0.045)	3.08
50		0.079 (0.065-0.094)	0.048 (0.040-0.059)	1.65
60		0.073 (0.057-0.098)	0.041 (0.035-0.049)	1.78
		0.061 (0.046-0.075)	0.041 (0.028-0.060)	1.49

* Each result is from a 2+2 assay design with at least three comparisons; values in parentheses are the 95 per cent fiducial limits of the mean potencies.

ous routes, to the presence of amino-acids derived from hydrolysis of the hormonal peptide, because Noble, Rinderknecht and Williams⁵ had shown that amino-acids enhance the antidiuretic effects of subcutaneously injected vasopressin. Noble *et al.*⁵, however, found that an amino-acid concentration of about 1 per cent was necessary to demonstrate this effect and this would represent a concentration of the vasopressin used by Fraser ('Post-lobin-V') of 2,000 U/ml., whereas Heller's² incubation mixture contained only 0.5 U/ml.

In view of the recent findings of Zaoral *et al.*¹ we decided to reinvestigate the action of mild alkaline treatment on synthetic arginine vasopressin (provided by Dr D. R. Chambers of Hoechst, Ltd.). The peptide (1.15 mg) was dissolved in 5 ml. of 0.05 normal acetic acid and this served as the stock solution. For each experiment, 1 ml. stock solution was diluted to 10 ml. with a carbonate-bicarbonate buffer of pH 10 (made by diluting 10 ml. of molar sodium carbonate and 20 ml. of molar sodium bicarbonate to 500 ml.) and a sample was immediately placed in the cold until assayed. The remainder of the solution was distributed in 1 ml. portions among ampoules which were then sealed and heated in a boiling-water bath for the required time. At the end of the heating period the ampoules were cooled and stored in the refrigerator until assayed—which in all cases was on the same day. Antidiuretic activity was determined by intravenous injection into rats according to a modification⁶ of the method of Jeffers, Livezey and Austin⁷. Vasopressor activity was also determined in rats by Dekanski's⁸ method. A commercial preparation of natural arginine vasopressin, 'Tonephin' (Hoechst AG, Frankfurt), was used as standard in all assays.

Table 1 shows that heating at pH 10 does cause a dissociation of the antidiuretic and pressor potencies of arginine vasopressin even when the former is measured by an intravenous method. Heating the hormone for periods of 30 min or longer increased the ratio of antidiuretic to vasopressor activity from approximately 1:1 to just under 2:1, although the absolute amount of each activity was considerably reduced. This result is compatible with the formation of some [8-D-arginine]-vasopressin as one of the reaction products. The ten-fold difference between antidiuretic and vasopressor potencies of heated hormone found by subcutaneous antidiuretic assay^{2,4} may be due in part to a delayed absorption caused by the amino-acids derived from hydrolysis of the hormone⁴, but perhaps more likely to the slower absorption of a modified hormone.

We thank Miss Anne Coxall for technical assistance.

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Received September 1; revised September 18, 1967.

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