experiments, a second injection given on the sixth day increased the habituation rate again.

Further purification of the transfer factor was done by treating the dialysate with 20 vol. of cold acetone. The precipitate was redissolved in water and injected into mice. Table 1 shows that this fraction was active when amounts equivalent to 140–340 mg of brain per mouse were given. The control preparation was inactive at 500 mg.

The transfer factor was found to be soluble in water, insoluble in acetone and 95 per cent ethanol. It is dialysable and, on partition with an equal volume of 88 per cent phenol, it goes into the phenol phase. These properties suggest that it is a peptide or small protein. This has been further confirmed by the disappearance of the activity when the preparation was incubated with crystalline chymotrypsin (1 mg/ml. at *p*H 8 for 1 h at room temperature). A similar incubation with pancreatic ribonuclease at *p*H 7 did not affect the activity.

Transfer of habituation has been described in *Planaria*[5], but, to our knowledge, there is no reference in the literature to the possibility of transfer of any type of learning in higher invertebrates or in vertebrates. The results summarized here suggest that information is recorded and stored in the nervous system in terms of protein structure. Most chemical theories of learning and memory emphasize the role of RNA in information coding[6] and it is probable that persistence of the protein code requires some self-replicating mechanism, through the nucleic acids.

The preliminary experiments reported here show that an elementary form of learning, habituation to sound, can be transferred to untrained animals by injecting them with a peptide-type material extracted from the brain of habituated animals. This factor is absent from the brain of non-habituated animals. Further experiments are in progress in other learning situations, other routes of introduction of the transfer factor and with extracts of circumscribed areas of the brain.

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Effect of Calcium and Other Ions on Vasopressin Release from Rat Neurohypophyses stimulated electrically *in vitro*

The rat posterior pituitary *in vitro* secretes vasopressin in response to a rise in extracellular potassium concentration. This effect of excess potassium is inhibited by magnesium and sodium ions and is strongly dependent on calcium. Secretion is abolished by omitting calcium from the incubation medium; it increases with increasing calcium concentration over a wide range, and is accompanied by uptake of calcium-45. From such evidence it has been concluded that calcium entry into the neurosecretory terminals on their depolarization by action potentials is the normal physiological event initiating secretion[1–3]. This concept is supported by the demonstration of vasopressin release from rat[3,4] and guinea-pig[5] neurohypophyses *in vitro* in response to electrical stimuli and of action potentials recorded from the supra-optico-hypophyseal tract[6]. It would clearly strengthen the hypothesis if it were shown that the effects of calcium and other ions on vasopressin release, elicited by electrical stimulation, were similar to their effects on secretion elicited by excess potassium. This communication presents evidence that this is so.

In each experiment, neurohypophyses from five male Sprague-Dawley rats (200–350 g) were dissected out and incubated in bicarbonate Locke's solution at 37° C and stimulated at 40-min intervals for 5 min at a frequency of 20 shocks/sec using an adaptation of the procedure already described[2] that allowed the five glands to be stimulated simultaneously. Vasopressin released into the medium was assayed on the rat blood pressure.

In 53 experiments the mean 'resting' release of vasopressin between the 30th and 40th min after setting up the preparation was $1{\cdot}48 \pm 0{\cdot}35$ (*S.E.*) mμ/5 glands/10 min. Electrical stimulation for the first 5 min of the succeeding 10-min period raised the mean output in this period to $18{\cdot}79 \pm 3{\cdot}3$ mμ/5 glands/10 min. This effect could be abolished reversibly by introducing 0·1 per cent procaine into the medium, and was therefore probably due to the production of electrical activity in the neurosecretory fibres.

The secretory response to electrical stimulation was completely abolished when the calcium concentration in the incubation medium was lowered to about 0·1 mM; it increased with increasing calcium concentration until this reached about 4 mM; and diminished as the calcium concentration was further raised to 8 or 16 mM. The curve relating vasopressin output in response to electrical stimulation to calcium concentration, obtained from experiments on 22 groups of glands, closely resembled that obtained previously using excess potassium to evoke secretion[2].

An increase in the magnesium concentration of the medium from 1 to 10 mM approximately halved the output of vasopressin in response to electrical stimulation. This effect could be reversed by raising the calcium concentration of the medium ten-fold.

Reduction of the sodium chloride concentration of the medium from 160 to 10 mM (tonicity being maintained with sucrose) enhanced vasopressin output. Thus in five sets of glands stimulated in the low sodium medium vasopressin output was $44{\cdot}2 \pm 7{\cdot}1$ mμ/5 glands/10 min while the corresponding control values from the same sets of glands in the high sodium medium was $22{\cdot}5 \pm 1{\cdot}7$.

The effects of calcium, magnesium and sodium on vasopressin output in response to electrical stimulation thus closely parallel their effects on neurohypophyses stimulated with excess potassium[1,2]. The experiments thus lend further support to the hypothesis that the sequence of events involved in stimulus-secretion coupling in the neurosecretory terminals of the hypothalamo-hypophyseal tract may be: the arrival of impulses, depolarization, and the entry of calcium ions.

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Acute Toxicity of Extracts of Morning-glory Seeds in Mice

The presence of alkaloids derived from lysergic acid in the seeds of the morning-glory varieties *Ipomoea violacea* (L.) and *Rivea corymbosa* (L.) Hall f. was discovered by Hofmann[1] and confirmed by several workers[2–5]. Reports of alteration of mental state after ingestion of morning-glory seeds have been made to this Directorate and have