The experiments here described contribute little toward the answers to two interrelated questions raised by our previous findings, i.e., what is the mechanism of enhanced poliovirus replication in fused systems of resistant cells? Are all the cells in the unfused systems we have studied completely resistant to this agent? It was originally postulated that in the presence of fusing cells poliovirus particles might be mechanically incorporated within the newly forming polynucleate complexes. The results presented in Table I afford further evidence that enhanced viral replication in the hamster cell system is dependent upon cell fusion, since the infectivity titers vary directly with the degree of fusion observed microscopically. These data, however, leave the manner in which the virus is introduced into the fusing complex still unclear. This might occur as we originally imagined, by physical incorporation of contiguous virions within the merging cytoplasm of individual cells, or as a result of focal impairment of the mononucleate cell membrane by the fusion factor or some other viral constituent, thus opening a way for the virus. A third mode of virus incorporation is suggested by the consistent finding in these as well as earlier experiments that a slight to moderate increase in poliovirus titers occurs in unfused systems. This increase may represent replication of the virus in a few susceptible cells or depend upon adsorption to resistant cells of viral aggregates in the original inoculum which subsequently are slowly dispersed. Which of these alternatives is correct must await further experimentation. However, the incorporation of such virus-associated cells, regardless of the nature of the association, into the syncytium might presumably serve to initiate the widespread infection observed.

The factors underlying the observed persistence of poliovirus over prolonged periods in both fused and unfused systems in slowly decreasing concentration, have also not yet been defined. Although the prolonged recovery of virus is *a priori* most readily attributable to its replication in a decreasing number of cells, preliminary results of experiments now in progress fail to support this hypothesis. We therefore intend to investigate the possibility that the slow dispersion of cell-associated aggregates may be responsible here as well as in the early apparent increase in titer found in unfused cell cultures.

Summary. Poliovirus can replicate and be passed serially in monolayer cultures of a line of hamster embryo cells subsequently fused with Sendai virus completely inactivated by low concentrations of beta propiolactone. Poliovirus fused into such a system produces a characteristic cytopathic effect.

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Intracranial Action of Oxytocin on Sodium Excretion by Conscious Dogs* (32669)

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Oxytocin is natriuretic in conscious dogs when the rate of urine flow is relatively low (1,2). Brooks and Pickford (1) found that small doses injected into the carotid circulation were natriuretic whereas doses 10–20 times as large were needed to produce com-

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parable responses if given intravenously. These observations suggest that oxytocin can act intracranially to influence sodium excretion. Brooks and Pickford made these observations on one dog with an exteriorized carotid loop. We have confirmed their findings and have also found that small doses of oxytocin injected into the third cerebral ventricle are natriuretic in conscious dogs.

Materials and Methods. Two groups of female mongrel dogs, weighing between 14 and 20 kg, were used. The first consisted of three trained dogs, each with its left common carotid artery exteriorized in a sleeve of skin, a carotid loop. The second group were five trained dogs with permanent cannulas implanted in their third cerebral ventricles (3).

Dogs were given 10–15 ml/kg of water by stomach tube 2–3 hours before urine collection was started. Bladder urine was collected through an indwelling catheter at 10-min intervals. Sodium and potassium were estimated by internal standard flame photometry. The hormones used were partially purified natural arginine vasopressin (Pitressin of bovine origin) kindly supplied by the late Dr. D. A. McGinty of Parke, Davis, and synthetic oxytocin (Syntocinon) supplied by the late Dr. R. Bircher of Sandoz Pharmaceuticals.

Results. Oxytocin injected into the carotid loop of a conscious dog produces clear natriuresis. In general, it takes about 1/10–1/5 of the effective intravenous dose to cause natriuresis when injected into the carotid artery. In five of seven trials in three dogs intracarotid oxytocin was natriuretic in doses between 0.6 and 1.2 mU/kg (Table I). In dogs in a similar state of hydration doses of 6–7 mU/kg intravenously are required to produce clear natriuresis (2).

Vasopressin is natriuretic when administered intravenously to dogs in water diuresis (1,2). When doses of 0.025–0.05 mU/kg were administered into the carotid loops of dogs hydrated by the administration of 40–50 ml/kg water they produced mild and transient natriuresis and antidiuresis. Antidiuresis suggests that the injected hormone had gained entrance into the systemic circulation and was acting directly on the kidneys.

These results support the observations of Brooks and Pickford (1) that oxytocin, in doses ineffective intravenously, increases sodium excretion in dogs with low rates of urine flow if injected into the carotid artery. Vasopressin, on the other hand, does not have a clearly demonstrable central natriuretic effect.

Brooks and Pickford (1) also reported that vasopressin appears to antagonize the central natriuretic action of oxytocin if the peptides were injected simultaneously into the carotid artery. They used a mixture of 20 oxytocic mU of oxytocin and 1 vasopressor mU of vasopressin. When we injected such a mixture into the carotid arteries of conscious dogs with low urine flow rates we observed clear natriuresis in seven of eight trials (Table I). The responses appeared quite similar to those in the same dogs after intracarotid oxytocin alone. The addition of vasopressin may have diminished the percentage increase in sodium excretion but, since these dogs had higher resting rates of sodium output, the meaning of this is not clear. In the dogs receiving both vasopressin and oxytocin there was a greater increase in potassium excretion, although this may not be significant.

Oxytocin was injected into the third cerebral ventricles in 12 trials in five dogs. The hormone was diluted in van Dyke–Hastings solution (4) with a final pH of 7.2–7.4. The total volume injected was 0.15–0.25 ml. No natriuresis was observed after injection of 0.1–0.3 mU/kg. In 4 of 8 trials, however, 0.6 mU/kg produced marked and prolonged natriuresis (Fig. 1). This dose injected into the carotid can occasionally produce a milder natriuresis with a much briefer duration (Table I). This dose is ineffective intravenously.

Natriuresis occurring after intraventricular injection was very slow in onset, appearing only 30 or 40 min after injection. In one case the natriuresis persisted for more than 2 hours (Fig. 1). The dogs showed no evident discomfort following intraventricular injections. Injections of equivalent volumes of solvent did not influence sodium excretion.

TABLE I. Responses to Intracarotid Injections of Oxytocin and Mixtures of Oxytocin and Arginine Vasopressin in Conscious Dogs.

		Doses (mU)		Urine flow[a]		Sodium excretion[a]		Potassium excretion[a]	
Dog	Weight	Oxytocin	Vaso-pressin	Before (ml/min)	Ratio (after/before)	Before (μeq/min)	Ratio (after/before)	Before (μeq/min)	Ratio (after/before)
A	15.5	9.4	0	0.45	0.67	13.0	2.31	12.0	0.54
B	17.5	10	0	0.21	0.90	26.5	0.81	2.2	0.86
B	17.5	11	0	0.30	0.67	25.0	1.80	6.2	1.19
B	17.5	20	0	0.17	1.06	3.0	2.70	7.7	1.30
C	16.5	20	0	0.15	1.00	12.7	1.73	7.1	1.06
C	16.5	20	0	0.20	1.25	12.5	1.84	3.0	1.50
C	16.5	20	0	0.40	0.87	9.5	1.16	15.2	1.25
Means $\pm$ SE				0.27	0.92 $\pm$ 0.08	14.6	1.76 $\pm$ 0.24	7.6	1.10 $\pm$ 12
A	15.5	9.4	0.47	0.15	0.67	17.3	1.29	8.6	2.33
B	17.5	20	1	0.25	1.00	50.0	1.31	7.0	1.50
B	17.5	20	1	0.30	0.67	46.0	1.24	8.0	1.13
B	17.5	20	1	0.35	0.86	37.5	1.29	6.5	1.23
B	17.5	20	1	0.20	1.50	31.0	1.01	7.5	1.40
C	16.5	20	1	0.33	1.42	21.2	1.46	7.0	1.16
C	16.5	20	1	0.37	0.73	16.2	1.68	6.2	0.94
C	16.5	20	1	0.25	0.80	11.5	2.13	3.0	1.33
Means $\pm$ SE				0.28	0.96 $\pm$ 0.07	28.8	1.43 $\pm$ 0.13	6.7	1.38 $\pm$ 0.15

[a] Figures shown represent average values for three 10-min periods prior to injection and three periods following injection.

Discussion. Our results are consistent with the hypothesis of Brooks and Pickford (1) that oxytocin acts intracranially to produce natriuresis. The location of the receptors involved has not been determined. Brooks and Pickford (1) ruled out the carotid body and sinus. Responses to oxytocin injected into the third ventricle favor the possibility that the hypothalamus or more caudal areas of the brain may be the site of action. The slow onset and long duration of the natriuresis in response to intraventricular oxytocin may be due to slow diffusion of the peptide into the responsive area. *In vitro* studies indicate that dog cerebrospinal fluid does not inactivate neurohypophysial peptides (5).

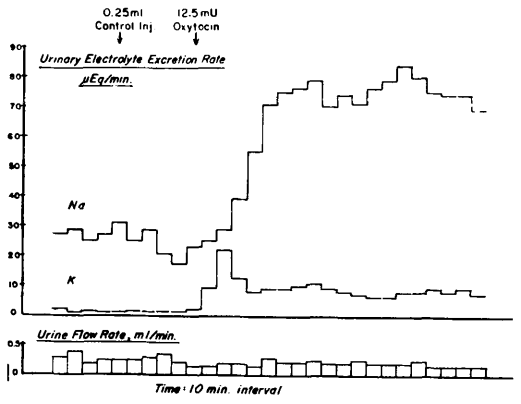

FIG. 1. Natriuretic response to oxytocin injected into the third cerebral ventricle of a conscious dog weighing 18 kg. An equivalent volume of solvent was injected at the first arrow.

Although these experiments support the suggestion that oxytocin acts centrally to produce natriuresis in dogs they do not indicate how this is brought about. Oxytocin may act to excite central structures that secondarily influence sodium excretion, either by changing the rate of secretion of a natriuretic or antinatriuretic hormone, or by more direct nervous influences on renal function. Földi *et al.* (6) have presented evidence based on crossed circulation experiments in anesthetized dogs that the natriuretic effect of whole posterior pituitary extract injected into the

carotid circulation depends largely upon the nervous connections between the head and the trunk. On the other hand Cort (7), working with cats, has adduced evidence that there is a hormone released from the hypothalamus that is directly natriuretic. The present experiments provide no answer to the important question of exactly how the central nervous system may exert its influence on natriuresis.

On the basis of these experiments we cannot speculate on the details of the renal response that are manifest as saluresis. We did not measure glomerular filtration rate or renal plasma flow. Brooks and Pickford (1) found that these variables did not change after intracarotid oxytocin. We (2) have not seen consistent changes after intravenous oxytocin. Small increases in filtration rate, however, could account for the additional sodium excreted and still escape detection by clearance measurements.

Summary. Doses of oxytocin that are ineffective intravenously produce natriuresis when injected into the carotid arteries or the third cerebral ventricles of conscious dogs. These observations appear consistent with the hypothesis that oxytocin exerts its natriuretic effect by acting upon some intracranial structure rather than directly upon the kidneys.

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Inhibition of the Chronic Ethanol-Induced Fatty Liver by Antioxidant Administration* (32670)

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A new concept of the mechanism of acute ethanol-induced hepatic injury was proposed following the demonstration that antioxidant administration either prior to, or simultaneous with ethanol, effectively inhibited hepatic triglyceride accumulation and ethanol-corn oil induced hypertriglyceridemia (1–5). As a result of these observations and the known role of antioxidants as inhibitors of peroxidative lipid deterioration, an hypothesis was advanced that lipid antioxidants exerted their protective effects by preventing an ethanol-induced free radical chain reaction which would lead to the formation of lipid peroxides, hydroperoxides, and/or other complexes (1–7). More recent studies (6,7) have demonstrated increased peroxidation of hepatic lipids following *in vivo* or *in vitro* administration of ethanol. The enhanced lipid peroxidation *in vivo* occurred prior to the accumulation of triglycerides (6,7), but was not observed with brain homogenates which do not metabolize ethanol, denoting that the lipoperoxidation process may be related to ethanol metabolism, rather than ethanol, per se (7).

Porta and Hartroft (8) have confirmed

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