Table I. Effect of ibenzmethyzin hydrochloride on H.Ep. No. 3 in conditioned rats

Dose: mg/kg s.c. × 8	C/T	% survivors	% weight change
100	4.4	50	− 32
50	1.7	80	− 22
25	1.5	100	− 5
Controls		90	+ 7

Table II. Effect of ibenzmethyzin hydrochloride on H.Ad. No. 1 in hamsters

Dose: mg/kg s.c. 14	C/T	% survivors	% weight change
60	2.7	20	− 20
30	2.3	80	− 6
15	0.9	80	+ 13
Controls		100	+ 33

In the case of H.Ad. No. 1, activity was also seen at a toxic dose of 60 mg/kg subcutaneously with 20% of the hamsters surviving and a weight loss of 20%. However, in addition, activity was also seen at a dose of 30 mg/kg subcutaneously where a slight weight loss occurred but where the majority (80%) of the animals survived. At a well tolerated dose of 15 mg/kg subcutaneously, where weight gain in addition to appreciable survival was noted, the substance was inactive.

Zusammenfassung. Ibenzmethyzinhydrochlorid zeigte tumorhemmende Wirkung gegen Human Epithelioma No. 3 in Ratten, die mittels Bestrahlung und Cortison konditioniert waren, und in Hamstern gegen Human Adenoma No. 1.

E. Grunberg and H. N. Prince

Department of Chemotherapy, Hoffmann-La Roche Inc., Nutley (New Jersey, USA), January 20, 1966.

An Attempt to Produce Antibodies to Oxytocin and Vasopressin

An attempt was made to produce antibodies to the neurohypophyseal hormones. In view of the fact that a bradykinin-albumin conjugate had been found to possess antigenic properties[1], we sought to conjugate oxytocin and lysine-vasopressin with a molecule of considerably larger size in order to investigate whether such compounds could induce antibody-production and whether the antibodies so produced would or would not inactivate the corresponding free hormones.

The oxytocin-albumin conjugate and the lysine-vasopressin-albumin conjugate were obtained by condensing oxytocin or lysine-vasopressin with rabbit serum albumin in the presence of a water-soluble carbodiimide: A solution of 40,000 I.U. of oxytocin or 20,000 I.U. of lysine-vasopressin in 50 ml dilute acetic acid was adjusted to pH 1.0 with methanesulphonic acid. The solution was concentrated to half its volume in vacuo at 25 °C to eliminate the acetic acid. The pH was then adjusted to 4.0 with 2*N* sodium hydroxide, and 40 mg of rabbit serum albumin was added. When the albumin had completely dissolved the pH was raised to 6.5 by further addition of 2*N* sodium hydroxide, and 400 mg of 1-cyclohexyl-3(2-morpholinyl-(4)-ethyl)-carbodiimide metho-*p*-toluene-sulphonate dissolved in 1 ml water was added. The mixture was stirred for 1 h at room temperature and dialysed for 48 h at 10 °C against distilled water in a Visking bag. The contents of the dialysis bag were lyophilized, yielding 25 to 50 mg of a white fluffy powder.

Oxytocin-albumin conjugate or lysine-vasopressin-albumin conjugate obtained as described above was administered to rabbits according to the following procedure in order to induce antibody formation: An injection of the antigen in Freund's complete adjuvans was administered subcutaneously and three weeks later a second injection was given by the intraperitoneal route. The dose of antigen per injection ranged from 2 to 6 mg, and a second course of injections was given to animals responding to the first course.

To demonstrate the presence or absence of antibodies to the antigens in question in the serum, a gel-diffusion test (double diffusion in two dimensions) for the detection of precipitating antibodies[2,3] was used. The presence of specific antibodies could be demonstrated, as is evident from Figures 1 and 2. Serial dilution tests, as shown in Figures 3 and 4, revealed that the antibody to oxytocin-albumin conjugate still gave precipitation patterns in a dilution of 1:16, whereas the titre for the antibody to vasopressin-albumin conjugate was somewhat lower (1:8).

To ascertain the specificity of the antibodies in question a certain number of control experiments was carried out. These are summarized in Table I. Oxytocin-albumin conjugate (OCO) and lysine-vasopressin-albumin conjugate (VCO) were selectively precipitated. No precipitation patterns were observed on testing the oxytocin or lysine-vasopressin conjugates on control sera from rabbits immunized with the following: rabbit serum albumin reacted with carbodiimide (AC), bovine serum albumin (BSA) or homologous serum albumin (RSA). Sera containing antibodies to the conjugates did not precipitate oxytocin (450 I.U./ml) or lysine-vasopressin (96 I.U./ml).

Antibodies to oxytocin-albumin conjugate, as well as to vasopressin-albumin conjugate, could also be demonstrated by the passive hemagglutination technique with tannic acid treated and with the antigen coated sheep red blood cells[4].

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[4] S. V. Boyden, J. expl. Med. *93*, 107 (1951).

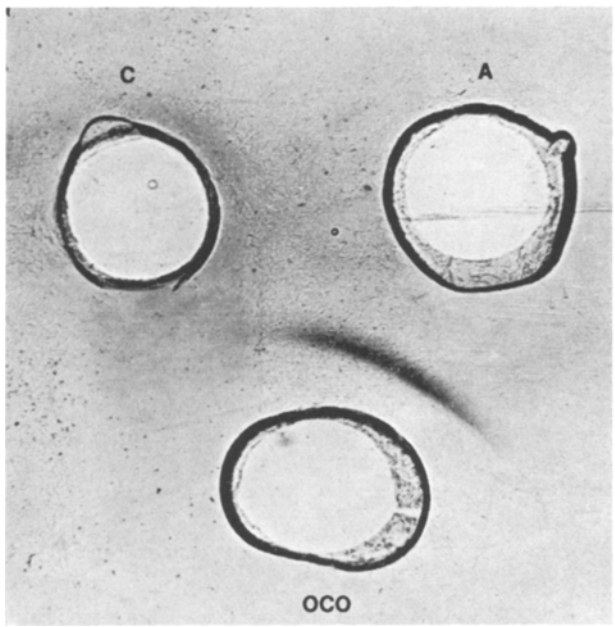

Fig. 1. Gel diffusion test, plate technique. OCO = oxytocin-albumin conjugate; A = serum from a rabbit immunized with oxytocin-albumin conjugate; C = serum from a control rabbit.

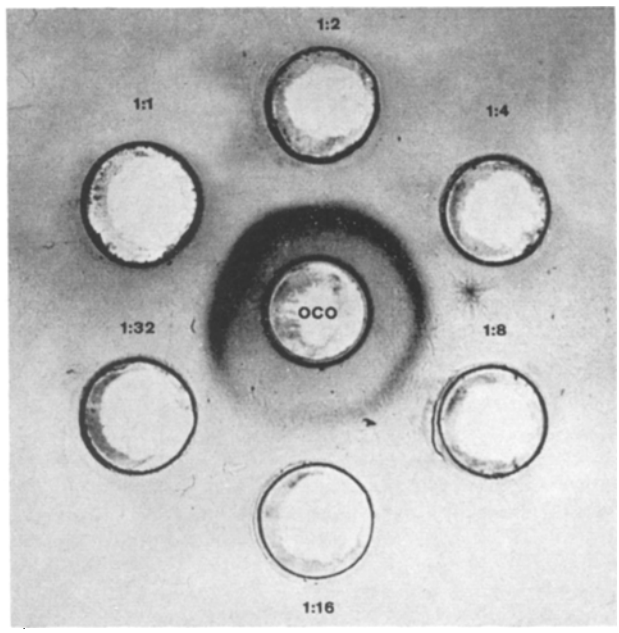

Fig. 3. Gel diffusion test, plate technique. In the centre oxytocin-albumin conjugate (OCO) and in the peripheral wells decreasing concentrations of serum from a rabbit immunized with oxytocin-albumin conjugate.

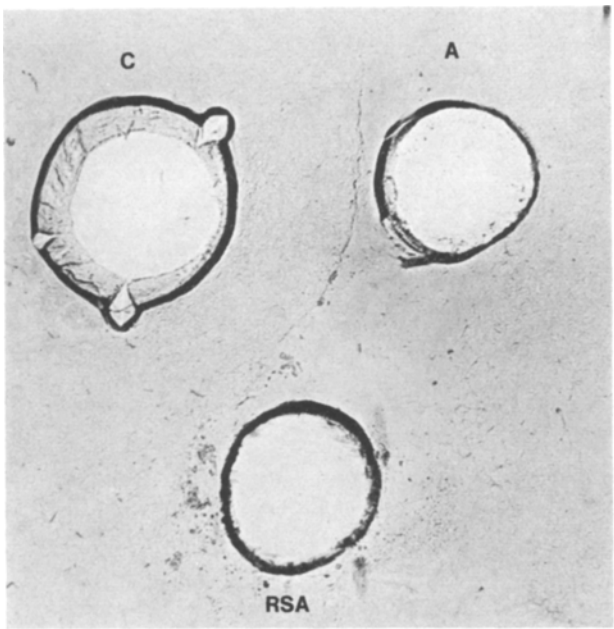

Fig. 2. Gel diffusion test, plate technique. RSA = rabbit serum albumin; A = serum from a rabbit immunized with oxytocin-albumin conjugate; C = serum from a control rabbit.

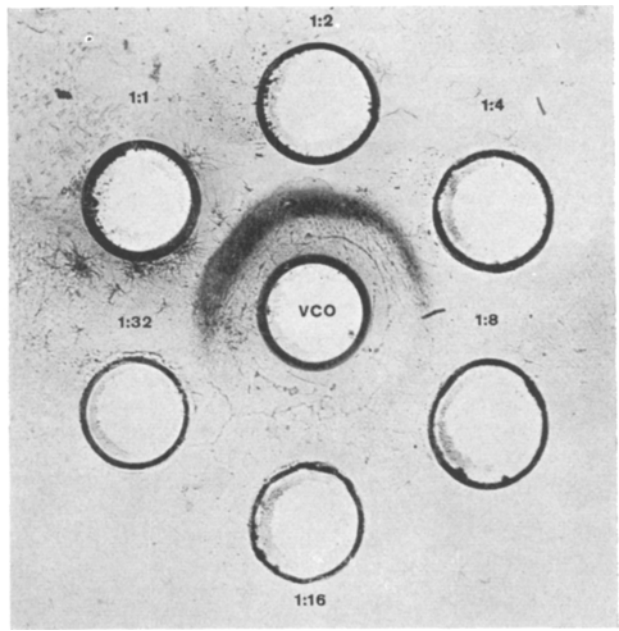

Fig. 4. Gel diffusion test, plate technique. In the centre vasopressin-albumin conjugate (VCO) and in the peripheral wells decreasing concentrations of serum from a rabbit immunized with lysine-vasopressin-albumin conjugate.

To investigate whether the antibodies produced by oxytocin-albumin conjugate were capable of inactivating oxytocin itself, synthetic oxytocin (Syntocinon) was incubated with undiluted or diluted serum containing antibodies to oxytocin-albumin conjugate at + 4 °C for 61 to 68 h. Similar incubation experiments were carried out with lysine-vasopressin and sera containing antibodies to lysine-vasopressin-albumin conjugate. Control experiments were run simultaneously with sera from rabbits which had not been immunized and which therefore did not contain antibodies to the hormone conjugates. At the end of the incubation period the proteins were precipitated by heating the solutions for 5 min at 95 °C in a water bath. Immediately afterwards the preparations were cooled and centrifuged. The supernatant was decanted off and its hormone content assayed.

Table I. Investigation of the specificity of the antibodies

Sera obtained from rabbits immunized with	Substances against which the sera were tested in gel-diffusion test							
	Oxytocin-albumin conjugate (OCO)	Lysine-vasopressin-albumin conjugate (VCO)	Albumin reacted with carbodiimide (AC)	Bovine serum albumin (BSA)	Rabbit serum albumin (RSA)	Oxytocin 450 I.U./ml	Lysine-vasopressin 96 I.U./ml	Freund's complete adjuvans
Oxytocin-albumin conjugate (OCO)	+	0	0	0	0	0	0	0
Lysine-vasopressin-albumin conjugate (VCO)	0	+	0	0	0	0	0	0
Albumin reacted with carbodiimide (AC)	0	0	0					
Bovine serum albumin (BSA)	0	0		+	+			0
Rabbit serum albumin (RSA)	0	0			0			
Freund's complete adjuvans	0	0						

+ = precipitation; 0 = no precipitation.

Table II. Oxytocic activity on the oestrous rat uterus in vitro of serum plus oxytocin mixtures after incubation at + 4 °C

Experiment No.	Serum of rabbit	Incubation			Activities found	
		Milieu		*Time, h*	Residual activity after incubation in % of Syntocinon originally added	Direct comparison: Activity after incubation with serum containing specific antibodies in terms of the activity measured after incubation with control serum, the latter activity being taken as 100%
		Serum concentration in %	Syntocinon concentration in I.U./ml			
1	Immunized	98	1.00	68	30.6 ± 2.6[a]	64.3 ± 8.4
	Control	98	1.00	68	53.6 ± 2.9[a]	
2	Immunized	98	0.50	63	25 ± 2	87 ± 5.8
	Control	98	0.50	63	28 ± 5	
3	Immunized	33	0.33	62	60 ± 4.9[a]	68.5 ± 16.6
	Control	33	0.33	62	80.6 ± 9.0[a]	
4	Immunized	33	0.1	61	55.3 ± 4.8	95 ± 5.9
	Control	33	0.1	61	64.4 ± 10	

[a] $p < 0.001$; in the other experiments $p > 0.05$.

Table III. Antidiuretic activity of serum plus lysine-vasopressin mixtures after incubation at + 4 °C

Experiment No.	Incubation			Direct comparison: Activity after incubation with serum containing specific antibodies in terms of the activity measured after incubation with control serum, the latter activity being taken as 100%
	Milieu		Time, h	
	Serum concentration in %	Lysine-vasopressin concentration in I.U./ml		
1	98	1.00	68	~100
2	98	0.50	63	87 ± 18
3	33	1.00	62	73 ± 16
4	33	0.33	61	106 ± 22

Residual oxytocin activity was assayed on the isolated oestrous rat uterus[5,6], 10 μg/l methysergide (Deseril) being added to the physiological solution, in order to exclude the influence of serotonin. Oxytocic activity was assayed against a solution of synthetic oxytocin (Syntocinon) as reference standard. In addition, mixtures of oxytocin and serum from rabbits immunized with oxytocin-albumin conjugate were compared directly with similar mixtures of oxytocin and serum from control animals. The rat uterus preparation was chosen in preference to the chicken blood pressure method to detect oxytocin, since histamine and catecholamines do not contract the oestrous rat uterus, and the effect of serotonin can be abolished by a suitable antagonist. Plasmakinins may, of course, have interfered to some extent.

In the lysine-vasopressin experiments the residual hormone was assayed from its antidiuretic activity on the diuresis of water-loaded rats in alcohol anaesthesia[7-9]. This test was chosen in preference to the rat blood pressure assay, since the antidiuretic activity of the vasopressins is highly specific. It was assumed that the high dilutions employed would eliminate any possible effects due to other substances, such as plasma-kinins, serotonin, histamine, for in this experimental set-up such substances only influence diuresis in relatively high doses. In all the experiments on vasopressin, residual antidiuretic activity after incubating vasopressin with sera containing specific antibodies to vasopressin-albumin conjugate was compared with the residual activity after incubating vasopressin with serum from control animals.

The data pertaining to oxytocin and lysine-vasopressin are summarized in Tables II and III respectively. The data in Table II suggest – but do not prove – that the antibodies produced by oxytocin-albumin conjugate may inactivate oxytocin itself to some extent, at least in certain conditions. This would not appear to hold for lysine-vasopressin (Table III).

The results reported here show that specific antibodies can be produced to oxytocin-albumin conjugate and lysine-vasopressin-albumin conjugate. The antibodies produced by oxytocin-albumin conjugate appeared to have some inactivating effect on oxytocin itself. Those produced by lysine-vasopressin-albumin conjugate did not inactivate lysine-vasopressin.

Zusammenfassung. Mit einem Oxytocin-Albumin-Konjugat bzw. mit einem Lysin-Vasopressin-Albumin-Konjugat konnten bei Kaninchen spezifische Antikörper hervorgerufen werden. Die gegen Oxytocin-Albumin-Konjugat gerichteten Antikörper scheinen auch einen gewissen inaktivierenden Effekt auf genuines Oxytocin zu haben.

L. P. HOLLÄNDER*, J. FRANZ**, and B. BERDE***

Blood Transfusion Centre of the Swiss Red Cross, Basle, Pharmaceutical Chemical** and Biological and Medical*** Research Division, Sandoz Ltd., Basle (Switzerland),*
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Postnatal Variations of the Dry Mass of Neuronal Nuclei of the Spinal Cord in Rat and Guinea-Pig[1]

The behaviour of the volumes of the nerve cell nuclei of the cerebral cortex and of the spinal cord has been studied in histological sections by SUGITA[2], NGOWYANG[3], and PETERS and FLEXNER[4]. The nuclei of the nerve cells can be obtained free in aqueous medium after homogenization of the tissue (VIOLA and PUCCINELLI[5]). With this method it is possible to distinguish nervous from glial nuclei[6] and to determine their exact shape[7] and dry mass by means of the Baker-Smith interference microscope[5].

Using this technique, a comparative study has been made of the volumes and dry mass variations of the nervous nuclei of the lumbar enlargement of the spinal cord in 0–150-day-old rats and guinea-pigs (Table).

The nuclei of the new-born rat have a dry mass and volumes 37% lower than those of 150-day-old rats. After birth both dry mass and volume increase for up to 20 days. The volume and the dry mass do not increase in a synchronous way. 5 days after birth only the dry mass is increased; as a consequence the total solid concentration increases. 20 days after birth the concentration reaches the value of the adult rat. After 20 days no nuclear varia-

Variations of the dry mass and volumes of the neuronal nuclei of the spinal cord (lumbar enlargement) in the rat and guinea-pig with age. 50 determinations were made for each age

Age (days)	Rat			Guinea-pig		
	Dry mass pg	Volume μ^3	Concentration pg/μ^3	Dry mass pg	Volume μ^3	Concentration pg/μ^3
0	38.0 ± 3.1	407.5 ± 31.1	0.096 ± 0.005	55.5 ± 2.5	658.7 ± 45.7	0.087 ± 0.008
5	41.8 ± 1.5	381.8 ± 19.3	0.127 ± 0.004	51.0 ± 2.1	642.9 ± 44.7	0.084 ± 0.009
10	57.1 ± 2.5	473.6 ± 25.7	0.125 ± 0.004	48.5 ± 5.7	698.8 ± 97.6	0.085 ± 0.006
20	64.0 ± 2.4	727.9 ± 38.5	0.091 ± 0.008	54.0 ± 3.0	699.0 ± 67.5	0.084 ± 0.005
150	59.9 ± 3.2	700.1 ± 58.6	0.090 ± 0.002	68.6 ± 3.3	1013.8 ± 69.8	0.079 ± 0.004