The antidiuretic activity of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin is qualitatively (phenotypically) similar to that of arginine-vasopressin. It differs from that of lysine-vasopressin in that it sets in more gradually and dies away more slowly (see, for example, [22]). The dose-response curves of arginine-vasopressin and deamino[1]-phenylalanine[2]-arginine[8]-vasopressin run parallel, so that the higher activity found for deamino[1]-phenylalanine[2]-arginine[8]-vasopressin may be considered to be generally valid and not merely confined to a particular dose range. The selectivity of the antidiuretic effect of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin, if contrasted with its vasoconstrictor activity, is, however, 27.5 times higher than that of arginine-vasopressin. The relatively weak effect on vascular smooth muscle is not restricted to one species, as the same low pressor activity was found both in spinal cats and in rats. Nor is this low activity on smooth muscle confined to vascular smooth muscle: its contractile action on intestinal smooth muscle, as evident from rabbit ileum assays, is weaker still.

It is also noteworthy that, whereas arginine-vasopressin possesses considerable uterotonic, avian depressor and milk-ejecting potencies, deamino[1]-phenylalanine[2]-arginine[8]-vasopressin shows hardly any oxytocin-like activities. Its oxytocin-like effect on the chicken blood pressure is atypical and indeed in this test it antagonizes oxytocin to a certain extent. To obtain 50% inhibition, it is necessary to inject 20 to 60 times more deamino[1]-phenylalanine[2]-arginine[8]-vasopressin than oxytocin.

The high degree of selectivity of the antidiuretic action of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin compared with its vasoconstrictor effect could hardly have been predicted: Although the omission of the phenolic group was known to increase the selectivity of the antidiuretic effect of arginine-vasopressin, this modification has been observed to have the opposite effect, i.e. to increase the selectivity of the pressor activity in the case of lysine-vasopressin, lysine-vasotocin, arginine-vasotocin, ornithine[8]-vasopressin and ornithine[8]-oxytocin. Suppression of the terminal amino group enhances antidiuretic selectivity in the case of arginine-vasopressin, lysine-vasopressin, ornithine-vasopressin, ornithine-oxytocin, oxytocin, phenylalanine[2]-ornithine-vasopressin, phenylalanine[2]-ornithine-oxytocin and phenylalanine[2]-oxytocin, albeit to a lesser extent than in the case of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin. It seems therefore that the nature of the shift in pharmacological profile brought about by a relatively minor chemical modification depends not only on the alteration itself but also on the general structure of the molecule. It is also evident from these results that our present knowledge concerning the receptor sites involved in the antidiuretic and pressor activities of the neurohypophysial hormones is still rather incomplete and that predictions on structure/activity relationships can only be made within narrow limits.

Zusammenfassung. Deamino[1]-Phenylalanin[2]-Arginin[8]-Vasopressin, dessen Synthese und pharmakologische Haupteigenschaften beschrieben werden, zeichnet sich durch eine hohe antidiuretische Wirkung aus. Dieser Effekt ist demjenigen des menschlichen antidiuretischen Hormons qualitativ ähnlich, jedoch wesentlich selektiver.

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Induction of Antibody Response in Lymphoid Cells in vivo and in vitro by RNA-Immuno-Carrier from Immune Serum

In previous papers the presence of a RNA carrier of the antibody template in sera of immunized rabbits (RNA-immuno-carrier or RNA-IC) was reported: this RNA-IC extracted from the serum of immunized animals is able to elicit in normal animals a precocious antibody response to the same antigens used for immunizing the animal source of RNA-IC[1-5].

Since it is known that the second phase of the antibody production occurs in the lymphoid tissues[6], we tested, in the present investigations, the influence of RNA-IC from the serum of rabbits immunized with guinea-pig RBC on lymphoid cells in vivo and in vitro. In consideration of the importance of the thymus in the immune processes we tested also the action of the RNA-IC on thymic cells: in fact the problems concerning the thymus are several and very interesting because, although it is commonly assumed that this organ is of great importance for the normal development of the lymphatic tissue and for inducing the formation of immuno-competent cells, it is still doubtful whether the thymocites are able, by themselves, to produce antibodies[7-10].

Production of RNA-IC. Selected rabbits weighing about 2 kg, fed on a standard diet, were immunized by 6–8 intravenous injections of guinea-pig RBC at 4–6 day intervals (the average antibody titer obtained was 1:3200). The RNA-IC from the immune sera was extracted by the phenol method described by Chargaff[11] and subjected

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to various controls as described in a previous paper[2]; it was lacking in agglutinating power towards guinea-pig RBC and contained less than 0.07% of total proteins. RNA-IC so obtained was used in the following experiments.

Induction of antibody formation by RNA-IC in vivo. 30-day-old Wistar rats were divided into three groups. The animals of the first group were kept as a control. To the animals of the second group were administered, by a single intracardiac injection, 2.5 mg/kg body weight of RNA-IC from anti guinea-pig RBC sera dissolved in a small volume of saline; these animals were then subdivided into three lots and sacrificed respectively 24, 48 and 72 h after RNA-IC injection; thymus and spleen were rapidly removed, washed with saline to remove every trace of blood, finely minced in a sterile petri dish, and suspended in medium 199 Difco (so that 1 ml of medium contained about 30 mg of tissue) in screw-cap tubes; after a brief shaking the tubes were left to stand for 10 min and the supernatant fluid withdrawn and used in the antibody plaque formation test. The animals of the third group were treated in the same way using RNA-IC preincubated with ribonuclease (50 μg for 0.25 mg RNA).

Induction of antibody formation by RNA-IC in vitro. Surviving cultures of normal rat thymocites were prepared in the following way: thymus of 30-day-old rats was treated as described above to obtain a suspension of free thymocites containing about one million cells per ml; with this suspension were prepared three groups of culture tubes, each tube containing 1.5 ml of cell suspension and 10,000 U of penicillin. The first group was kept as a control, to the second group was added 0.25 mg per tube of RNA-IC from anti guinea-pig RBC serum, and to the tubes of the third group was added the same amount of RNA-IC preincubated at 56°C for 30 min with 50 μg of ribonuclease (Sigma). After 48 h at 37°C the culture fluids were withdrawn, centrifuged at 20,000 *g* for 60 min, and used in the slide hemagglutination test and in the immunofluorescent test performed by the usual methods[2].

Antibody plaque formation test. Thymus and spleen cells obtained from the rats injected with RNA-IC were subjected to the antibody plaque formation test according to the technique of JERNE and NORDIN[12]: screw-cap tubes containing 0.75% agar were immersed in boiling water to melt the agar and then cooled to 40°C, to the agar were added thymus and spleen cells obtained as described above (1 million per ml) and guinea-pig RBC (200 millions per ml); the mixture was stirred and poured onto a supporting 1.4% agar bottom layer in a petri dish so as to form a thin, semisolid top layer; after incubation at 37°C for 1 h this layer was covered with 1.5 ml of complement; a further incubation of 1 h revealed several clear plaques of hemolysis. Controls were made with thymus and spleen cells from normal rats and from rats injected with ribonuclease-treated RNA-IC. Antibody plaque formation tests were made also with larger fragments of thymus and spleen collected after separation of the free cells: the clear

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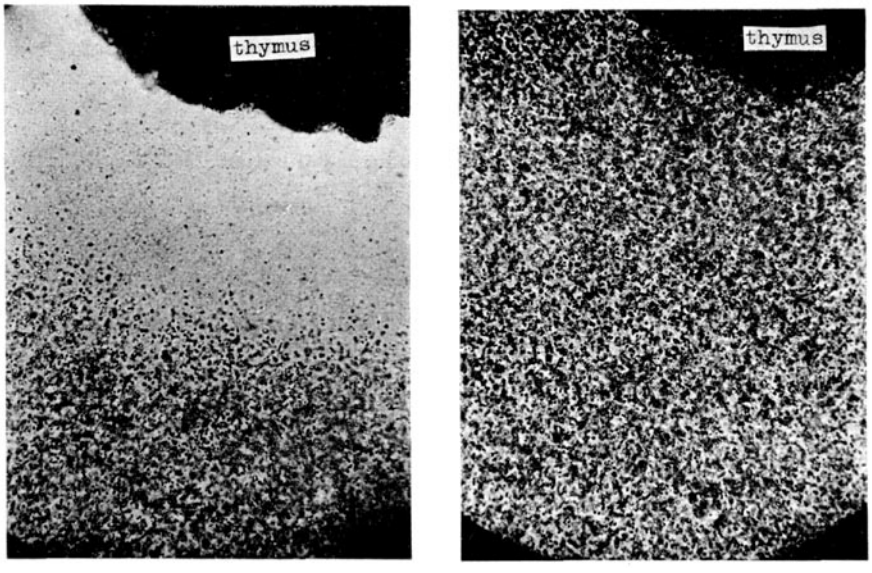
Table I. Number of hemolytic plaques per plate in the antibody plaque formation test with thymus and spleen cells from (i) control rats, (ii) rats injected with RNA-IC from anti guinea-pig RBC serum and (iii) rats injected with ribonuclease treated RNA-IC

h after injection	Control rats (injected with saline)		Rats injected with RNA-IC		Rats injected with ribonuclease-treated RNA-IC	
	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen
24	0	1	37[a]	6	3	0
48	1	0	25[a]	17[a]	1	1
72	0	2	8	42[a]	0	1

[a] Highly significant ($P < 0{,}01$). These data represent the average of three experiments rounded to nearest number.



Left: hemolytic area around a small fragment of thymus from rat injected with RNA-IC of anti guinea-pig RBC serum. *Right:* no hemolysis around a small fragment of thymus from rat injected with ribonuclease-treated RNA-IC.

Table II. Titers of anti-guinea-pig RBC antibodies in culture fluids of (i) normal rat thymocites, (ii) thymocites treated with RNA-IC from anti guinea-pig serum and (iii) thymocites treated with RNA-IC preincubated with ribonuclease

Titers	Slide hemagglutination test			Immunofluorescence test		
	Thymocites alone	Thymocites + RNA-IC	Thymocites + ribonuclease-treated RNA-IC	Thymocites alone	Thymocites + RNA-IC	Thymocites + ribonuclease-treated RNA-IC
1:2	—	++++	—	—	++++	—
1:4	—	++++	—	—	++++	—
1:8	—	+++	—	—	++++	—
1:16	—	±	—	—	+++	—
1:32	—	—	—	—	—	—

plaques of hemolysis in this case were very large and evident.

Results. Thymus and spleen cells of rats injected with RNA-IC from anti guinea-pig RBC serum, showed several hemolytic plaques in the antibody plaque formation test in comparison with the same cells from normal rats or from rats injected with ribonuclease-treated RNA-IC. The number of hemolytic plaques was greater in the thymus than in the spleen of the animals sacrificed 24 h after RNA-IC injection, about the same in the animals sacrificed 48 h after injection, and greater in the spleen than in the thymus of the animals sacrificed 72 h afterwards (Table I). Similar results were obtained with small thymus and spleen fragments (Figure 1). The culture fluid of rat thymocites, which had been in contact for 48 h with RNA-IC from anti guinea-pig RBC serum, had agglutinating power towards the same RBC at a dilution of 1:12, and the immunofluorescent test gave positive results till a dilution of 1:25. Culture fluids of normal thymocites or of thymocites which had been for 48 h in contact with ribonuclease-treated RNA-IC gave always negative results (Table II).

Conclusions. The presence in the serum of immunized animals of a RNA carrier of the antibody template capable of eliciting a rapid antibody response in vivo and in vitro is confirmed. The point of action of this RNA-IC is found in the lymphoid cells both in vivo and in vitro. In the animals injected in vivo with RNA-IC, specific antibodies appear rapidly in the thymus and rapidly disappear: the decrease of antibody production in the thymus corresponds to an increase in the spleen.

Riassunto. Nel siero di animali immunizzati è presente un RNA depositario del modello anticorpale che è capace di indurre la formazione di anticorpi in cellule linfoidi normali in vivo ed in vitro, sia di origine timica che splenica.

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Studies on the Action of some Enzymes on the Cyst Wall of Isolated Metacerkariae[1] from the Liver Fluke, *Fasciola hepatica* L.[2]

The wall of the cyst of *Fasciola hepatica* consists of an outer strongly eosinophilic and an inner weakly eosinophilic layer[3]. Recent studies[4–6] have revealed that both layers are subdivided into two strata. Thus the outer layer of the wall consists of a spongy proteinaceous and an inner more fibrous predominantly mucopolysaccharidic layer. The inner layer of the cyst wall again contains an outer mainly mucopolysaccharidic layer and an inner fibrous proteinaceous layer possibly covered by lipids. The occurrence of phenolic substances in the cyst wall has also been pointed out[7]. At excystment the young fluke emerges through an opening in the inner layer. This excystment is induced in vitro by some added proteolytic enzymes but also by external systems devoid of enzymes[3].

As some enzymes act fairly specifically on their substrates, a series of enzymes of different origin and with different substrate specificity were chosen in the following analytical study of the cyst wall. The effect of the enzymic action was studied with light and electron microscopy.

Metacerkariae were obtained according to a method by Boray[8]. Ten of them were incubated at 38°C in small chambers containing 0.2 ml of 0.1 *M* phosphate buffer, pH 7.0 or 0.01 *M* HCl, pH 2.0, together with 0.2 mg of the active enzymes listed in the Table. Others were incubated without addition of enzymes and represented the controls. After 3 h of incubation the metacerkariae were

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