the cell does not treat the structures as foreign to it. The antibodies must have passed the cell membrane unaltered which is, however, only possible if the normal function of the cell membrane has been damaged[7].

Except in nephroblastomas, all authors mention the rare occurrence of the described intracytoplasmic structures in various tumour cells. In the nephroblastomas they are often observed, and only in the podocytes of the glomerulus and especially in the less differentiated cells[3]. The importance of the podocytes for the normal functioning of the kidney and the effects which a change in their permeability has, is known[8]. Swelling of the mitochondria and damage of the cristae in the podocytes of kidney tumours have been described[3]. Localization, morphology and function of these cells form the preconditions of an intracytoplasmic immune precipitation in vivo, under the pathological conditions described.

The morphology of antigen-antibody complexes suggests antibody formation against virus structures, ribosomes and ribosome mRNA. That the ribosomes appear smaller after pepsin digestion is explained by the breaking of RNA from the ribosomes surface[9], whose organization was recently newly formulated[10].

The immune aggregates have hitherto only been observed in systems that also permitted the presence of antibodies; a precondition that is also given by cells in vitro. Besides the natural occurrence of antibodies in the medium, immune competent small lymphocytes are often present in cultures of myeloblastose virus infected myeloblasts of chickens[11]. The only known exception was found in chicken cells infected by Rous sarcoma virus in vitro in a heterologous medium[12]. Apart from one rather uncertain exception[13], no Rous sarcoma virus neutralizing factor has been found up to now in the serum of mammals[14]. The first observation of the intracytoplasmic enclosures was made in tumours of the hatched chickens, produced by high doses of Rous sarcoma virus[15].

Zusammenfassung. Die durch Viren der Vögel-Sarkom-Leukose-Gruppe hervorgerufenen intrazytoplasmatischen Strukturen sind sehr wahrscheinlich Immunpräzipitate.

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Blockade of Ovulation in the Rat Initiated by an Antimetabolite

3-Acetylpyridine (3-AP) and several structurally related antimetabolites'[1] interfere with the coenzyme function of pyridine nucleotides by competing with nicotinamide for sites within the molecule[2,3]. The toxicity of 3-AP to mammals is to a certain extent determined by the dietary intake of nicotinamide, nicotinic acid or tryptophane[1,3] and is neutralized, in the acute experiment, by equimolar amounts of nicotinamide[1] or diphosphopyridinenucleotide (NAD)[3].

Coenzymes of the nucleotide type play a role in ovarian steroidogenesis in mammals, but it is controversial whether they directly mediate the steroidogenic action of luteinizing hormone (LH)[4,5] or whether the conversion of steroids observed in their presence is independent of LH[6,7].

In this study, 3-AP has been found to interfere with ovulation in the intact rat if administered at appropriate times during vaginal pro-oestrus. Although there is no evidence for a specific effect of 3-AP on ovarian pyridine nucleotides (as opposed to those located in other body compartments), a fairly high degree of temporal specificity with respect to reproductive events is achieved by the rapidity of interchange between 3-AP and nicotinamide on the one hand and by the well-defined 'critical period' for LH release in rats exposed to controlled conditions of illumination[8] on the other.

3-AP[9] was diluted 1:100 v/v with distilled water and injected i.p. into adult female rats of the Wistar strain at various times during pro-oestrus. The animals were exposed to 14 h of light and 10 h of darkness and fed a standard diet containing 2.5 mg nicotinic acid/kg. All exhibited regular 4-day cycles as assessed by daily vaginal smears. 3-AP was administered at a dose level of 0.6 ml (approximately 54 μmoles)/100 g body weight. This dose was close to the LD_{25} when used without subsequent antidote. Ovulation was assessed by counting tubal ova during the following morning (oestrus).

Tubal ova were absent in all cases in which the antimetabolite was administered 3 h or more prior to the onset of the 'critical period' (Table I). In view of this, 3-AP was injected at − 4 h in all subsequent experiments. The blocking effect of 3-AP was counteracted by equimolar doses of nicotinamide, irrespective of whether this

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Table I. Blocking effect of 3-AP in relation to 'critical period' for LH release

Time of injection (h)	No. of eggs (mean ± S.E.)
− 6	0 (17)
− 4	0 (11)
− 3	0 (13)
− 2	2.6 ± 1.0 (7)
− 1	7.2 ± 2.1 (6)
0	7.0 ± 1.5 (6)
+ 2	6.7 ± 0.8 (11)
+ 4	11.0 ± 0.9 (4)

0 h denotes start of critical period. Numbers in parentheses indicate No. of animals on which each figure is based.

Table II. Influence of nicotinamide, NAD and NADP on 3-AP blockade

Nicotinamide (low dose: 54 µmoles per 100 g body weight)	Nicotinamide (excess: 100 mg/rat)	NAD (50 mg/rat)	NADP (20 mg/rat)	Eggs produced (mean ± S.E.)
− 4[a]				7.7 ± 0.7 (3)
− 3				8.3 ± 1.2 (3)
− 2				6.8 ± 2.5 (4)
− 1				8.4 ± 0.9 (5)
− 1/2				8.7 ± 1.2 (3)
0				7.7 ± 1.4 (3)
+ 1/2				0 (3)
	− 4[a]			10.3 ± 2.2 (3)
	− 3			9.7 ± 0.9 (3)
	− 2			11.7 ± 1.9 (3)
	− 1			10.7 ± 0.9 (3)
	− 1/2			12.5 ± 0.6 (4)
	0			2.3 ± 1.2 (3)
	+ 1/2			0 (3)
		− 4[a]		10.3 ± 1.3 (3)
		− 1		9.3 ± 1.2 (3)
		− 1/2		0 (2)
		0		0 (2)
			− 4[a]	8.0 ± 2.9 (3)

[a] Time of administration (h). 0 h denotes onset of critical period. 3-AP was administered at − 4 h in all cases. Numbers in parentheses indicate No. of animals for each figure.

Table III. Ovarian sensitivity to exogenous LH

µg LH (i.p.)	No. of eggs/animal Nembutal blockade	3-AP blockade
100	11	7, 5, 0
50	12, 12	8, 3, 0
40	14, 14	4, 0, 0
20	10, 13	4, 5, 5, 7
15	11, 8	4
10	11, 11	0
5	10, 3, 7, 9, 4, 14	0, 0
0	0 (42 rats)	0 (20 rats)

antidote was administered simultaneously or up to 3 1/2 h after the injection of the antimetabolite (Table II). However, an excess of nicotinamide was required for the production of the full set of eggs. NAD and NADP, administered in doses calculated to contain less than equimolar amounts of nicotinamide, also exhibited a considerable protective effect (Table II). The injection of 3-AP alone drastically reduced the ovarian response to exogenous LH: whereas animals of the same colony blocked by Nembutal[8] (35 mg/kg body weight 30–45 min before the start of the 'critical period') consistently shed a full set of ova in response to 10 µg of bovine LH, the response of animals blocked with 3-AP and injected with doses up to 100 µg was erratic and hardly ever complete (Table III). Animals treated with Nembutal alone predictably ovulated with a delay of 24 h[8], whereas no ova were found at this time in rats blocked with 3-AP.

The reduced sensitivity of the ovary to exogenous LH following the administration of 3-AP suggests an ovarian site of action of the antimetabolite. (An affinity of 3-AP for certain hippocampal neurones has been described[10,11], and the hippocampus has been implicated in ovulation, at least in the rabbit[12]. However, histological sections of the relevant hippocampal areas have not revealed any signs of morphological damage resulting from the experimental design used in this study.) The ready reversibility of the ovarian blockade by nicotinamide, NAD or NADP indicates a requirement for pyridine nucleotides in certain biochemical steps, presumably steroidogenic in nature, leading to ovulation. Progesterone is known to be secreted in increased amounts in rat ovarian venous effluent during the afternoon of pro-oestrus[13], and the rabbit releases 20-α-hydroxypregn-4-en-3-one within minutes following coitus or the administration of LH[14].

These results support the view that the action of LH is mediated, at least in part, by pyridine nucleotides and that ovulation and ovarian steroidogenesis are interdependent events. The selective inhibition of ovulation at this level would require agents of greater specificity, comparable to that exhibited by metopirone in the adrenal gland[15,16].

Zusammenfassung. Verabreichung von 3-Acetylpyridin zu bestimmten Zeiten der Pro-oestrus-Phase hemmt die Ovulation bei der Ratte. Dieser Hemmeffekt wird durch Pyridinnukleotide antagonisiert.

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