Since it has been shown that injury to the median eminence or blocking of dopamine receptors by haloperidol prevents the effect of calcitonin on prolactin secretion [4] it can be tentatively suggested that an important role in the mechanism of negative feedback between prolactin secretion and the blood calcitonin concentration is played by dopamine, and that this connection is closed in the hypothalamus (at the level of the median eminence).

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CHANGES IN PLOIDY CLASSES OF LEFT ATRIAL MYOCYTES AFTER LIGATION OF THE LEFT CORONARY ARTERY IN RATS

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Stimulation of DNA synthesis in myocytes of the left atrium after infarction have been clearly demonstrated [5]. A recent cytofluorometric study of atrial myocytes revealed a considerable increase in the mean number of binuclear cells 11 days after ligation of the left coronary artery [9].

The aim of this investigation was to study the effect of this procedure at different times after the operation.

EXPERIMENTAL METHOD

The left cornary artery was ligated in male Wistar rats weighing 120-140 g at the point of its origin from the aorta [10]. Some rats of the same weight were left intact as controls, and on some animals a mock operation was performed — the pericardium was removed. The animals were killed with ether after 10, 20, 50, 90, and 150 days. The auricle of the left atrium was fixed in 10% formalin solution in buffer at pH 7.0 and dissociated with alkali into single cells [2]. Feulgen's reaction was carried out on films of isolated cells. The DNA content in the nuclei was determined on a Vickers M-86 scanning microdensitometer. The number of mono- and binuclear monocytes (in %) was determined accurately by examining a further 2000-3000 cells from each animal.

EXPERIMENTAL RESULTS

Intact control rats weighing 150 g had diploid (2c) myocytes in the left atrium 10 days after the beginning of the experiment, although binuclear cells were found in all films, mainly with diploid nuclei (2c × 2), together with a fraction of a per cent of multinuclear and other myocytes with high levels of ploidy (Table 1; Fig. 1a). After 40 or 80 days the number of binuclear myocytes (2c × 2) in intact rats weighing about 300 g rose to 23-24% (Fig. 1b), in agreement with data in the literature [9]. In three of four animals which survived 10 days after ligation of the left coronary artery, the composition of the myocytes

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TABLE 1. Changes in Weight of Rat and of Its Heart and Composition of Myocytes from Auricle of Left Atrium after Ligation of Left Coronary Artery or Removal of Pericardium

No. of rats	Experimental conditions	Time from beginning of experiment, days	Weight of rats, g	Weight of fixed heart, mg	Ploidy class of myocytes			
					2c	2c×2	4c	high degree of ploidy
1, 2	Intact rats	10	135, 190	570, 710	90,0—92,3	7,0—9,5	0,1—0,4	0,1—0,6
3, 4, 5	Ligation	10	145, 148, 175	570, 580, 835	83,0—91,0	8,3—15,6	0,6—1,3	0,1—0,4
6	"	10	185	585	72,1	26,5	0,8	0,3
7, 8	Intact rats	50, 90	245, 345	760, 920	71,0—71,5	23,1—24,5	3,4—5,3	0,2—0,5
9, 10	Ligation	20	155, 170	580, 700	73,0—87,3	11,5—24,5	1,0—1,5	0,2—0,6
11	"	20	200	1130	61,9	37,3	0,6	0,2
12, 13, 14	"	50	275, 285, 345	1020, 1050, 1064	67,2—90,0	9,7—28,5	0,2—4,0	0,1—3,0
15	"	50	260	1080	12,6	52,4	19,1	15,9
16	"	90	325	1560	7,1	19,1	10,0	63,8
17	"	150	380	1360	73,0	25,7	0,9	0,4
18	Removal of pericardium	10	155	620	78,6	20,2	0,8	0,4
19	"	10	183	600	49,3	46,3	3,0	1,4
20, 21	"	50	235, 270	720, 760	88,6—95,4	4,0—9,4	0,5—1,2	0—0,8

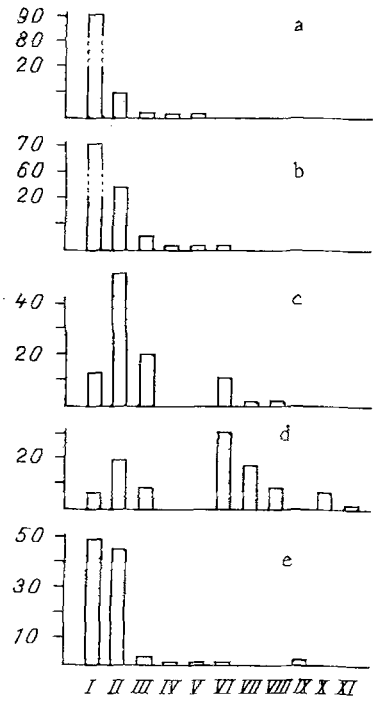

Fig. 1. Composition of myocytes from auricle of left atrium of rat heart according to ploidy. Abscissa, class of ploidy: I) 2c, II) 2c × 2, III) 4c, IV) 2c × 3, V) 2c × 4, VI) 4c × 2; VII) 8c × 2, IX) 4c × 4, X) 16c, XI) 16c × 2; ordinate, number of cells (in %). a) Intact rat, 10 days after beginning of experiment; b) intact rat, 40 days; c) ligation of left coronary artery, 50 days; d) ligation of left coronary artery, 90 days; e) mock operation, 10 days.

was the same as in the control rats of the same age (about 90% of diploid cells). In one rat in which the artery was ligated (No. 6, Table 1) an increase was observed in the number of binuclear cells up to a level characteristic of more adult rats, and of intact animals with greater weight. At the next stage of the experiments (20 days after ligation) the composition of the atrial myocytes in three rats also was heterogeneous: in one animal this parameter was virtually the same as in the control at the previous time, In another it was virtually the same as the control at the next stage (50 days after the beginning of the experiment), although this rat weighed less than the more adult rat; in a 3rd rat (No. 11, Table 1) the number of binuclear cells was greater than in the first two rats. In two rats which survived 50 days after the operation the composition of the myocytes was about the same as in control animals of the same age. In one animal the number of binuclear cells was the same

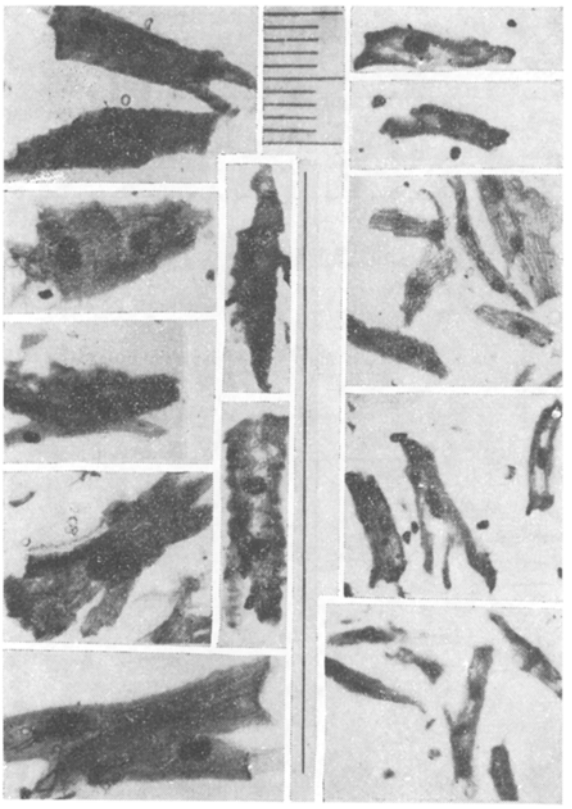

Fig. 2. Myocytes of left atrium in control (left) and after infarction (right). Myocytes in experimental hearts grossly hypertrophied and ploidy of their nuclei is increased. Azure-eosin. 250×.

as that even in much younger rats (9.7%). One rat undergoing ligation of the artery (No. 15; Table 1; Fig. 1c), in which 2c × 2 myocytes accounted for more than half of the population of these cells, and in which there were 19.1% of mononuclear tetraploid myocytes (about 4% in the control) and 15.9% of cells with high levels of ploidy (not more than 0.5% in the control), differed sharply from these animals; moreover, among these latter classes there were many 4c × 2 cells, and mononuclear octaploid cells, which were not found in general in intact rats of the same age and weight, and even cells with two octaploid nuclei, were found. Even greater individual differences were observed in rat No. 16 (Table 1; Fig. 1d), in which almost 64% of the cells were of high ploidy, i.e., the number of these cells was increased by more than 100 times. The modal class consisted of binuclear 4c × 2 myocytes, octaploid with respect to the total DNA content; a few 8c cells were present, as well as 8c × 2 and 16c cells, hexadecaploid as regards their DNA content, which were virtually not found in the remaining 20 rats. About 1% of the myocytes were of the 16c × 2 class (Fig. 2).

High incorporation of thymidine into the atrium myocytes after myocardial infarction [5] is not itself evidence of an increase in the number of cells. Polyploidization of myocytes was discovered in that investigation and also later [9]. Variability of this process in individual animals was demonstrated. In more than half of animals undergoing operation no change in the composition of the atrial myocytes in general was found. In the rest an increase in ploidy was observed, to a varied degree. Variability in polyploidization of atrial myocytes can evidently be explained by the relatively low hypertrophy of the heart in the majority of animals. According to Rumyantsev [6], a marked increase in DNA snythesis took place only in the left atrium of those hearts whose weight exceeded that of the control by 140-160% or, more especially, by 170-190%. In the present investigation only the heart of rat No. 16, with the most marked changes in ploidy of the myocytes, belonged to this last category. The composition of the myocytes did not correlate with changes in weight of the whole heart. In rat No. 15 (Table 1), for instance, with a marked change in composition of the myocytes, the heart weighed 1080 mg, whereas in the other three rats which underwent the same procedure, the hearts weighed about the same although the composition of their myocytes did not differ from the control. We know that postnatal growth of the ventricles of the

heart takes place mainly through an increase in weight of the individual cells outside the mitotic cycle [3]. The atria have not yet been studied, and the causes of the increase in weight of the heart in all rats undergoing the operation compared with its weight in control animals of the same age are not yet clear. The most significant changes in ploidy were observed after 50 and 90 days, but they could also take place earlier. The largest number of cells labeled with thymidine and the highest mitotic index were observed during the first 10 days after ligation of the left coronary artery [7], but polyploidization of cardiomyocytes can take place only through mitosis [1].

In two rats surviving 50 days after the mock operation of removal of the pericardium, and with the same body weight and weight of the heart as intact animals of the same age, the composition of the myocytes was the same as that of much younger rats. A marked increase in ploidy of the myocytes, far greater than in intact and older rats, took place in one rat only 10 days after removal of the pericardium (Fig. 1e). The number of binuclear 2c × 2 cells in this rat was almost 6 times greater than in intact animals. Data on stimulation, just as on inhibition of proliferation of atrial myocytes after removal of the pericardium, are important. Removal of the pericardium itself may affect the state of the myocytes, in particular because it leads to dilatation of the ventricles [8]. This effect and (or) decompensation were perhaps responsible for the decrease in thickness of the free wall of the left ventricle after ligation of the left coronary artery also.

Post mortem examinations have shown that postinfarction cardiosclerosis is often accompanied by hypertrophy of the left atrium [4]. To what extent hypertrophy of the left atrium can compensate for a disturbance of ventricular function, and in what cases growth of the cell genomes takes place will be made clear by future research. It is evident now that the response to similar actions is sufficiently heterogeneous and that proliferation can be stimulated by different actions.

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