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Alimentary Production of Gallstones in Hamsters

15. Production of gallstones under varied hormonal conditions I.*)

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With 5 tables

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A series of papers from this laboratory deal with the alimentary production of gallstones in hamsters and the influence of diet on the composition of the bladder bile in this and other species.

In the present communication we report a preliminary study on the alimentary development of gallstones under varied influence of hormones, intended to serve as an introduction to a more comprehensive investigation on this subject.

Experimental

The diet used for development of gallstones was the previously described diet "No. 295" containing 20% casein, 72.3% sucrose, and 2% lard (1). Given to young hamsters it produces cholesterol gallstones as well as amorphous pigmented, or mixed gallstones, but with somewhat varying proportions between the frequency of the individual types of stone from one set of experiments to another, a phenomenon which can be due to the nutritional prehistory of the animals.

The hamsters were young from our stock colony, 170 in number. They were started simultaneously on the gallstone producing diet at the age of 27–49 days, and kept in experiment for about two months as far as possible.

20 males and 20 females served as controls, i. e., they received no hormonal treatment.

10 other males were orchidectomized and 10 other females ovariectomized 6 days after the beginning of the gallstone producing dietary regimen.

The remaining 110 animals received hormonal treatments as described in the following, beginning 2–4 days after institution of the gallstone producing dietary regimen.

The following hormones were given by subcutaneous injection of stabilized suspensions of microcrystals (suitably diluted when necessary):

Testosterone iso-butyrate (Perandren, CIBA), 2.5 mg bi-weekly, corresponding to a total of 10 mg per animal during 42–56 days of treatment. 10 intact males underwent this treatment.

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Estradiol monobenzoate (Follicyclin, CIBA), 40 micrograms at intervals of 3 weeks, corresponding to a total of 120 micrograms per animal during 48–58 days of treatment; 10 intact females underwent this treatment.

Progesterone (Lutocyclin, CIBA), 16.6 mg bi-weekly, corresponding to a total of 66.4 mg per animal during 45–54 days of treatment. 10 intact females were treated in this way.

Deoxycorticosterone trimethylacetate (Percorten, CIBA), 2.5 mg at intervals of 3 weeks, corresponding to a total of 5 mg per animal during 38–41 days, or 7.5 mg per animal during 44–60 days of treatment. 10 males and 10 females were treated with deoxycorticosterone.

The following hormonal (or antihormonal) substances were given mixed with the diet from the third day of the experimental feeding period:

Cortisone acetate (Cortisone, CIBA, and Cortone acetate, M.S.D.), 2.5 mg%, furnishing 0.1 mg cortisone per animal per day with an estimated daily consumption of 4 g of diet. 10 males and 10 females received the diet with cortisone acetate.

Desiccated thyroid gland (with 0.21% iodine in organic combination), 0.02%, furnishing 0.8 mg of the desiccated gland per animal per day with a daily consumption of 4 g of diet. 10 males and 10 females received the diet with desiccated thyroid.

Methylthiouracil, 0.2%, furnishing 8 mg of the substance per animal per day with a daily consumption of 4 g of the diet. 10 males and 10 females received the diet with methylthiouracil.

Results and Discussion

The data for the individual animals in the various groups are presented in tables 1–4. A summary of the findings is shown in table 5.

For estimation of the significance (table 5, section 2) of the differences in the incidences of cholesterol gallstones as well as of the differences in the incidences of amorphous pigmented gallstones between two groups of animals, an animal having both types of stones is counted as an incidence of cholesterol stones and an incidence of amorphous pigmented stones. The same applies to an animal having mixed gallstones.

The estimations of significance were carried out by the method described by S. KOLLER (in the section "Beurteilung von Häufigkeiten" in reference 2).

The significances mentioned in the following would refer to 99.73% probability for non-randomness under the assumption that the animals were otherwise completely comparable[1]).

It is seen that the untreated and unoperated *control animals* have a rather high incidence of cholesterol gallstones and a low incidence of amorphous pigmented gallstones. There is no significant difference between the two sexes with respect to either type of gallstones[2]).

[1]) The last mentioned qualification is obviously not fulfilled, since, as seen from tables 1–4, all the animals were not killed after the same length of time; some of them died, some had diarrhea, whereas others had not. It is, however, a general experience that a difference in the duration of the experiment of a few weeks has no marked influence on gallstone production when the animals have been more than a month in experiment on diet no. 295. Further, diarrhea influences the results only by shortening the experimental period, since animals with diarrhea are always killed as soon as this condition is recognized. Treatment of the results as described gives at least an approximate estimate of their significance, and in the absence of more exact data such as chemical analyses of the calculi within each group it is the only way in which the results can be judged.

[2]) From the results obtained with a much larger number of animals it has been found that the incidence of amorphous pigmented gallstones is about twice as high among the

Table 1. Unoperated control animals. Data for individual animals.

Group number	Animal number	Sex[1])	Age in days at beginning of exp.	No. of days in exp.	Gall-stones[2])	Weight gain[3] max. g	Weight gain[3] final g	Diar-rhea	Found dead
453	106	m	44	56	O	22	22*)	+	+
453	34	m	43	58	C	15	11*)	+	
453	57	m	42	58	C	16	15*)	+	
453	65	m	42	58	A	17	17*)	+	
453	49	m	39	64	C	25	21		
453	108	m	44	64	C	60	60		
453	118	m	44	64	O	58	55		
453	152	m	31	64	O	48	48		
453	164	m	31	64	C	47	47		
453	42	m	44	64	A	38	35		
454	23	m	43	30	O	9	8*)	+	+
454	25	m	46	36	C	13	5	+	+
454	7	m	ca. 40	38	C	20	20*)	+	+
454	35	m	43	38	C	14	8*)	+	
454	41	m	43	38	C	17	17*)	+	
454	83	m	37	48	C	19	19*)	+	+
454	60	m	42	49	C	23	21*)	+	+
454	102	m	44	53	C	24	15*)	+	+
454	92	m	44	54	C	16	16*)	+	
454	73	m	44	54	CA	28	28		
461	59	f	43	54	C	16	16*)	+	+
461	1	f	45	63	O	8	−2		
461	16	f	42	63	C	15	15		
461	36	f	44	63	C	7	6		
461	37	f	44	63	C	8	6		
461	52	f	47	63	O	18	9		
461	58	f	43	64	O	10	7		
461	95	f	45	64	C	10	8		
461	116	f	45	64	C	10	10		
461	122	f	47	64	C	11	11		
462	4	f	45	26	O	17	17*)	+	+
462	18	f	45	28	C	8	8*)		
462	29	f	47	28	C	18	18*)	+	
462	54	f	45	28	C	13	13*)	+	+
462	76	f	47	28	C	21	21*)	+	
462	161	f	32	72	C	11	11*)		
462	174	f	32	64	O	17	17		
462	104	f	45	72	C	14	14*)		
462	112	f	45	72	A	23	23*)	+	+
462	154	f	32	72	O	8	6*)		

females as among the males, but this difference between the sexes does not necessarily manifest itself in smaller groups of animals, especially when the tendency to formation of this type of gallstones is low. Since in the present experiment, the female control animals had a lower incidence of amorphous pigmented gallstones than the male controls, the anding of a higher incidence of this type of gallstones in one of the other groups may fippear to be more pronounced in the females than in the males.

In the *gonadectomized animals* of both sexes, the incidence of cholesterol gallstones is lower and the incidence of amorphous pigmented gallstones higher than in the control groups of the same sex. If both sexes are considered as one group, the differences from the control group are significant with respect to the amorphous type of stones. The percentage of animals without gallstones was not altered by gonadectomy.

Table 2. Gonadectomized animals. Gonadectomy was performed 6 days after beginning of the experimental feeding. Data for individual animals.

Group number	Animal number	Sex[1])	Age in days at beginning of experim. feeding	No. of days in gonadectomized state	Gallstones[2])	Weight gain[3]) max. g	Weight gain[3]) final g	Diarrhea	Found dead
455	77	m	46	52	O	21	20*)	+	+
455	78	m	46	55	A	25	25*)	+?	
455	47	m	42	56	C	18	12*)	+	+
455	93	m	44	56	C	18	15*)		
455	11	m	43	57	A	18	15	+	
455	114	m	43	57	A	13	10	+	
455	17	m	41	59	C	25	25*)	+	
455	21	m	43	63	A	16	16*)	+	
455	126	m	43	62	O	22	20	+	
455	123	m	46	63	A	10	8*)	+	
463	82	f	38	47	O	7	2*)	+	+
463	24	f	47	51	O	6	6	+	
463	163	f	32	51	A	13	7	+	+
463	6	f	ca. 40	54	O	21	21*)		
463	97	f	45	54	C	9	9*)		
463	145	f	31	54	A	35	35*)	+	
463	162	f	32	54	CA	13	13*)	+	+
463	165	f	32	54	CA	12	12*)	+	
463	171	f	32	54	A	26	26*)	+	
463	175	f	30	54	A	30	30*)	+	

Table 3. Animals treated with hormones by subcutaneous injection. Data for individual animals.

1. *Testosterone isobutyrate.* 2.5 mg bi-weekly.
First injection 3 days after the beginning of the gallstone producing regimen.

Group No.	Animal No.	Sex[1])	Age in days at beginning of gallstone producing regimen	No. of injections received	No. of days under hormonal treatment	Gallstones[2])	Weight gain[3]) max. g	Weight gain[3]) final g	Diarrhea	Found dead
456	22	m	43	3	42	C	11	10*)	+	+
456	61	m	42	4	43	O	26	26*)	+	+
456	63	m	42	4	44	O	11	10*)	+	
456	85	m	44	4	44	O	22	22*)	+	
456	84	m	37	4	54	C	9	9	+	+
456	80	m	37	4	56	C	11	5*)		
456	87	m	48	4	56	C	17	17*)		
456	111	m	44	4	56	C	12	10*)		
456	143	m	30	4	56	C	9	7*)		
456	170	m	31	4	56	O	20	20*)		

2. *Estradiol monobenzoate*, 40 micrograms at intervals of 3 weeks.
First injection 4 days after the beginning of the gallstone producing regimen.

Group No.	Animal No.	Sex[1])	Age in days at beginning of gallstone producing regimen	No. of injections received	No of days under hormonal treatment	Gallstones[2])	Weight gain[3]) max. g	Weight gain[3]) flanal g	Diarrhea	Found dead
464	55	f	45	3	48	A	9	2*)	+	+
464	72	f	45	3	50	O	14	6*)	+	
464	67	f	46	3	52	O	11	−1*)	+	
464	9	f	44	3	57	C	5	0*)		
464	101	f	45	3	57	C	14	11*)		
464	113	f	44	3	58	C	7	7*)		
464	125	f	44	3	58	C	7	4*)		
464	129	f	45	3	58	C	12	12*)		
464	130	f	45	3	58	C	14	14*)		
464	173	f	32	3	58	C	13	13*)		

3. *Progesterone*, 16.6 mg bi-weekly.
First injection 3 days after the beginning of the gallstone producing regimen.

465	166	f	32	4	45	O	23	23*)	+	+
465	155	f	32	4	46	O	11	11*)	+	+
465	147	f	31	4	46	O	31	20	+	
465	124	f	44	4	53	A	31	31*)	+	+
465	10	f	44	4	54	A	40	40	+	
465	48	f	43	4	54	O	26	26		
465	64	f	43	4	54	A	26	26	+	
465	127	f	44	4	54	A	36	36		
465	133	f	43	4	54	M	39	39*)	+	
465	139	f	29	4	54	CA	24	24		

4. *Deoxycorticosterone trimethylacetate*, 2.5 mg at intervals of 3 weeks.
First injection 3 days after the beginning of the gallstone producing regimen.

457	30	m	46	2	38	C	21	21*)	+	+
457	44	m	44	2	40	C	31	31	+	+
457	103	m	44	2	41	O	14	4*)	+	+
457	128	m	43	3	44	O	27	27*)	+	+
457	81	m	37	3	60	O	18	16		
457	109	m	44	3	60	A	29	29		
457	119	m	46	3	60	C	18	16		
457	146	m	30	3	60	C	34	34		
457	149	m	30	3	60	C	11	7		
457	158	m	31	3	60	C	25	19		
466	5	f	45	3	58	C	12	12*)		
466	8	f	44	3	58	O	16	8*)		
466	15	f	42	3	58	C	16	16*)		
466	26	f	47	3	58	A	25	25*)		
466	39	f	44	3	58	C	14	13*)		
466	62	f	43	3	59	A	10	8*)		
466	70	f	42	3	59	A	24	24*)		
466	94	f	45	3	59	C	19	19*)		
466	144	f	31	3	59	O	20	17*)		
466	172	f	32	3	59	A	28	28*)		

Table 4. Animals treated with hormones (or antihormones) added to the gallstone producing diet from the third day of experimental feeding. Data for individual animals.

Treatment	Group No./ Animal No.	Sex [1]	Age in days at beginning of gallstone producing regimen	No. of days under hormonal treatment	Gallstones [2]	Weight gain [3] max. g	Weight gain [3] final g	Diarrhea	Found dead
Cortisone acetate, 2.5 mg%	458/32	m	43	60	C	17	17*)		
	458/68	m	41	63	C	32	32*)		
	458/71	m	44	68	C	31	30		
	458/105	m	44	67	C	27	27*)	+	+
	458/3	m	44	67	C	22	22*)	+	
	458/27	m	46	67	C	25	25*)		
	458/50	m	46	67	C	35	35*)		
	458/121	m	46	68	C	26	26		
	458/140	m	26	68	C	31	31		
	458/167	m	31	68	C	31	31		
	467/132	f	43	58	C	10	7*)		
	467/13	f	45	69	C	6	3		
	467/19	f	45	69	C	13	6		
	467/38	f	44	69	C	10	8		
	467/69	f	42	69	C	12	11		
	467/75	f	47	69	C	15	12		
	467/79	f	38	69	C	6	1		
	467/89	f	49	69	C	13	13		
	467/178	f	31	69	C	14	10		
	467/117	f	45	69	C	4	0		
Desiccated thyroid, 0.02%	459/100	m	44	46	O	35	35*)	+	+
	459/14	m	41	56	O	18	9*)		
	459/54	m	44	56	O	27	27*)		
	459/56	m	44	56	O	32	32*)		
	459/74	m	44	56	A	27	27*)		
	459/86	m	41	68	A	44	41		
	459/90	m	48	68	C	56	56		
	459/134	m	42	68	O	34	34		
	459/168	m	31	68	A	37	37		
	459/179	m	ca. 40	68	M	39	39		
	468/2	f	45	56	O	20	15*)		
	468/20	f	45	56	O	20	19*)		
	468/45	f	45	58	A	33	33*)		
	468/96	f	45	58	A	31	31*)		
	468/99	f	45	58	A	17	17*)		
	468/177	f	31	60	O	14	9*)	+	+
	468/115	f	45	69	O	5	−2		
	468/156	f	32	69	O	13	6		
	468/141	f	27	69	O	16	11		
	468/176	f	30	69	A	29	29		

Treatment	Group No./ Animal No.	Sex [1]	Age in days at beginning of gallstone producing regimen	No. of days under hormonal treatment	Gallstones [2]	Weight gain [3] max. g	Weight gain [3] final g	Diarrhea	Found dead
Methylthiouracil 0.2%	460/43	m	44	63	C	40	40	+	+
	460/40	m	43	65	C	9	8*)	+	+
	460/33	m	43	66	C	8	7*)	+	+
	460/131	m	44	67	C	29	29*)	+	+
	460/42	m	44	68	C	26	10	+	
	460/46	m	42	69	C	14	−8*)	+	
	460/110	m	44	69	C	16	12*)	+	+
	460/120	m	46	69	C	8	3	+	
	460/135	m	42	69	C	9	1	+	
	460/169	m	31	69	O	16	16		
	469/157	f	32	52	C	9	2*)	+	
	469/107	f	45	55	O	3	0	+	
	469/66	f	46	69	O	29	29		
	469/28	f	47	69	C	23	23		
	469/31	f	47	69	C	11	8		
	469/53	f	47	69	O	27	27		
	469/88	f	49	69	C	11	8		
	469/91	f	49	69	C	9	7		
	469/98	f	45	69	C	3	−1		
	469/150	f	31	69	C	20	20		

Footnotes to tables 1–4:

[1]) m = males; f = females.

[2]) C = cholesterol gallstones
M = mixed gallstones
A = amorphous pigmented gallstones
CA = cholesterol gallstones and amorphous pigmented gallstones occurring together
O = no gallstones

[3]) max = weight gain from beginning of the gallstone producing regimen until maximal weight has been obtained.
final = weight gain from the beginning of the gallstone producing regimen until the end of the experiment.

An asterisk after the figures means that the last weighing day was in the week before the day on which the animal was sacrificed (or died). Otherwise the weighing took place on the day of sacrifice.

Administration of *testosterone* to intact males and of *estradiol* to intact females, apparently had no significant influence on gallstone production under the circumstances of the experiment.

If the testosterone treated males and the estradiol treated females are considered as one group, the incidence of amorphous pigmented gallstones in these animals is significantly lower than the corresponding incidence in the gonadectomized animals.

Table 5. Summary of the experiment.

Group no.	Sex	Treatment	No. of animals	Section 1 Incidences of gallstones of the various types Number					Section 1 Percent					Section 2 M and CA counted as C and A Incidence, percent		f and m as one group No. of animals	f and m as one group Incidence, percent	
				C	M	A	CA	O	C	M	A	CA	O	C	A		C	A
453	m	None	10	5	0	2	0	3										
454	m	None	10	8	0	0	1	1	65	0	10	5	20	70	15	40	67.5	10
461	f	None	10	7	0	0	0	3										
462	f	None	10	6	0	1	0	3	65	0	5	0	30	65	5			
455	m	Orchidectomy	10	3	0	5	0	2	30	0	50	0	20	30	50	20	30	55
463	f	Ovariectomy	10	1	0	4	2	3	10	0	40	20	30	30	60			
456	m	Testosterone	10	6	0	0	0	4	60	0	0	0	40	60	0	20	65	5
464	f	Estradiol	10	7	0	1	0	2	70	0	10	0	20	70	10			
465	f	Progesterone	10	0	1	4	1	4	0	10	40	10	40	20	60			
457	m	Deoxycorticosterone	10	6	0	1	0	3	60	0	10	0	30	60	10	20	50	25
466	f	Deoxycorticosterone	10	4	0	4	0	2	40	0	40	0	20	40	40			
458	m	Cortisone	10	10	0	0	0	0	100	0	0	0	0	100	0	20	100	0
467	f	Cortisone	10	10	0	0	0	0	100	0	0	0	0	100	0			
459	m	Thyroid gland	10	1	1	3	0	5	10	10	30	0	50	20	40	20	10	40
468	f	Thyroid gland	10	0	0	4	0	5	0	0	40	0	50	0	40			
460	m	Methylthiouracil	10	9	0	0	0	1	90	0	0	0	10	90	0	20	80	0
469	f	Methylthiouracil	10	7	0	0	0	3	70	0	0	0	30	70	0			

C = cholesterol gallstones. M = mixed gallstones. A = amorphous pigmented gallstones. CA = cholesterol gallstones and amorphous pigmented gallstones occurring together. O = no gallstones. m = males. f = females. Two figures connected with a vertical line on their right-hand side are considered significantly different.

As a consequence of these results, future studies with testosterone and estradiol should be carried out by administration of the hormones to gonadectomized animals or to animals on a diet favoring the formation of amorphous pigmented gallstones[1]).

As a result of the treatment with testosterone, vesiculae seminales all were of normal size, whereas those in the orchidectomized animals all were atrophic. About half of the control males had atrophic testicles. The uteri of the estrogen treated females were of normal size, whereas those in the ovariectomized animals were atrophic. About half of the females in the control group had thin or atrophic uteri.

Intact females treated with *progesterone* had a lower incidence of cholesterol gallstones and a higher incidence of amorphous pigmented gallstones than the untreated female controls. The difference with respect to amorphous pigmented gallstones was significant.

Since, thus, the effect of progesterone on gallstone formation resembles that of ovariectomy, it is likely that ovariectomy influences the formation of gallstones either through the lack of estrogen or through the increased level of gonadotropic hormones.

Deoxycorticosterone showed no clear-cut effect on the incidence of gallstones.

All the animals treated with *cortisone* had cholesterol gallstones, and none of them had amorphous pigmented gallstones. Since the control groups have rather high incidences of cholesterol stones and low incidences of amorphous pigmented stones, the differences from the control groups are not significant. Apparently, future experiments with cortisone should be carried out under circumstances in which the untreated controls have a lower incidence of cholesterol gallstones and a higher incidence of amorphous pigmented gallstones.

Caira et al. (5) have reared hamsters on the basal diet used in the present study modified by omission of lard and vitamins A, D, E, and K. Thirty-eight animals of both sexes having received no other treatment were autopsied after 7 to 60 days. Twenty-nine of them had gallstones consisting mainly of cholesterol, but also calcium containing and amorphous precipitations occurred. Ten hamsters of each sex were given intraperitoneal injections of estradiol benzoate (5 micrograms per g body weight twice weekly for 28 days), whereas 10 hamsters of each sex were given intraperitoneal injections of progesterone (66 micrograms per g body weight once weekly for 28 days), and 10 hamsters of each sex received intraperitoneal injections of cortisone acetate (33 micrograms per g body weight once weekly for 28 days). The administration of estrogen, progesterone and cortisone was found to decrease the incidence of calculi formation, a finding which did not occur in our experiments. The calculi developed during estrogen and progesterone treatment were of the same character as those developed without hormonal treatment, whereas the calculi developed under cortisone treatment were almost pure cholesterol calculi.

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[1]) During the preparation of this paper we noticed two publications (3, 4) on the production of cholesterol gallstones in mice fed a low protein diet containing 1% cholesterol and 0.5% cholic acid. The gallstones appeared earlier in the female mice than in the males, and the flow rate of the hepatic bile was higher in the females than in the males. This sex difference could be reversed by treating the females with testosterone or the males with estrone. The doses used were: testosterone, 1 mg in aqueous suspension, intramuscularly every 3 days for 21 days; estrone, 1 mg in aqueous suspension, intramuscularly every 5 days for 20 days.

These latter statements agree with our observations only as far as estrogen and cortisone treatment are concerned. The duration of the hormone treatment was only half as long as ours, but the dosage with estradiol and cortisone was more intense. The interval between the injections chosen by CAIRA et al. corresponds approximately to the unusually short estrous cycle in this species of abaut 4 days (6). The omission of vitamin A is an unneccesary complication. Cortisone was found by CAIRA et al. to increase serum total cholesterol to about 190 mg % from about 115 mg % in the hamsters receiving the diet without hormone treatment.

The animals treated with *desiccated thyroid gland* had a lower incidence of cholesterol gallstones and a higher incidence of amorphous pigmented gallstones than the control animals. If both sexes are considered as one group, the difference from the control group is significant as far as cholesterol stones are concerned.

The animals treated with *methylthiouracil* had a high incidence of cholesterol gallstones and no amorphous pigmented gallstones, but these incidences are not significantly different from those in the control groups, even if both sexes are considered as one group. However, the incidence of cholesterol stones as well as the incidence of amorphous pigmented stones in the methylthiouracil treated animals are significantly different from the corresponding incidences in the animals treated with thyroid gland. It is, therefore, likely that the results obtained with desiccated thyroid gland are due to the thyroid hormone rather than to other components of this material.

Miss PRANGE has analyzed a large number of amorphous pigmented gallstones collected through several years from hamsters, most of which were reared on the basal diet used in the present study (7). The following constituents were found (percentage by weight):

Ca	12.1
Mg	0.5
Na	3.7
K	0.3
P	9.5
N	1.7
S	1.08
Glycine-conjugated bile acids	20.0
Taurine-conjugated bile acids	2.0
Fatty acids (mostly C_{16}, C_{18})	4.0
Biliverdin	2.0
Ash	46.3
Ether-extractable matter	0.8

These components account for about 80% of the weight of the stones. Proteins and, probably, mucopolysaccharides were also present, but their amounts were not determined.

The fact that the calcium salts of glycine-conjugated dihydroxycholanic acids are only sparingly soluble in water may be the cause of the presence of these compounds in the stones. However, glycocholic acid was also present.

It seems likely that certain changes in the composition of the bile which tend to stabilize the solubilization of cholesterol may involve increases in

concentrations of components entering into the composition of the amorphous pigmented gallstones and thereby increase the tendency to formation of such stones.

According to ERIKSSON (8), the total production of bile acids and the ratio chenodeoxycholic/cholic acid are higher in hyperthyroid than in hypothyroid rats. Similar changes in hamsters might be thought to favor solubilization of cholesterol and to further precipitation of material containing the sparingly soluble calcium chenodeoxycholate in the bile of the thyroid treated animals. However, HELLSTRÖM and SJÖVALL (9) have found (in man) that the ratio glycine conjugation/taurine conjugation is increased in the hypothyroid condition.

It is, therefore, not possible to suggest an explanation for the observed differences in formation of gallstones in hamsters fed desiccated thyroid gland and methylthiouracil, resp., until sufficient information about the composition (and perhaps also about the flow rate) of the bile of hamsters under these conditions has been obtained.

Explanation of the changes observed after gonadectomy or progesterone treatment must also await analyses of the bile.

All the experiments of the present study have been concerned with variations in the type of gallstones produced by a gallstone inducing diet under varied hormonal influence.

Future experiments should also tackle the problem whether a diet not causing gallstones in hormonally normal hamsters can prevent formation of gallstones under drastically altered hormonal conditions.

Summary

Young hamsters from our stock colony were reared on a diet with 20% casein, 72.3% sucrose, and 2% lard, which produced a rather high incidence of cholesterol gallstones and a low incidence of amorphous pigmented gallstones in the intact and untreated control animals.

After *gonadectomy* of both sexes, the incidence of cholesterol gallstones was lower and the incidence of amorphous pigmented gallstones higher than in the control animals, but the percentage of animals without gallstones was not altered by gonadectomy.

Under the circumstances of the experiment, *testosterone* treatment of the intact males and *estradiol* treatment of the intact females showed no influence on gallstone production.

Intact females treated with *progesterone* had a lower incidence of cholesterol gallstones and a higher incidence of amorphous pigmented gallstones than the untreated females.

All the animals treated with *cortisone* had cholesterol gallstones and none of them had amorphous pigmented gallstones.

Animals treated with *desiccated thyroid gland* had a lower incidence of cholesterol gallstones and a higher incidence of amorphous pigmented gallstones than the control animals.

Animals treated with *methylthiouracil* had a high incidence of cholesterol gallstones and no amorphous pigmented gallstones.

The principles for further studies on the alimentary production of gallstones under varied hormonal conditions are discussed.

Zusammenfassung

Junge Hamster von unserer Kolonie wurden mit einer Gallensteine hervorrufenden Nahrung gefüttert. Diese Nahrung, welche 20% Kasein, 72,3% Saccharose und 2%

Schweineschmalz enthielt, rief eine recht hohe Inzidenz von Cholesterin-Gallensteinen und eine niedrige Inzidenz von amorphen pigmentierten Gallensteinen in den intakten und unbehandelten Kontrolltieren hervor.

Nach Gonadektomie der beiden Geschlechter war die Inzidenz von Cholesteringallensteinen niedriger und die Inzidenz von amorphen pigmentierten Gallensteinen höher als in den Kontrolltieren. Der prozentuale Anteil von Tieren ohne Gallensteine wurde durch Gonadektomie nicht geändert.

Unter den benutzten Versuchsbedingungen zeigten Testosteronbehandlung der intakten Männchen und Oestradiolbehandlung der intakten Weibchen keinen Einfluß auf die Gallensteinbildung.

Progesteronbehandelte intakte Weibchen hatten eine niedrigere Inzidenz von Cholesteringallensteinen und eine höhere Inzidenz von amorphen pigmentierten Gallensteinen als die unbehandelten Weibchen.

Alle mit Cortison behandelten Tiere hatten Cholesteringallensteine, und keines von diesen Tieren hatte amorphe, pigmentierte Gallensteine.

Tiere, welche mit Gl. thyreoidea sicc. behandelt wurden, hatten eine niedrigere Inzidenz von Cholesteringallensteinen und eine höhere Inzidenz von amorphen pigmentierten Gallensteinen als die Kontrolltiere.

Tiere, die mit Methylthiouracil behandelt wurden, hatten eine hohe Inzidenz von Cholesteringallensteinen und keine amorphen pigmentierten Gallensteine.

Die Prinzipien für weitere Untersuchungen über die alimentäre Gallensteinbildung unter variierten hormonalen Bedingungen sind erörtert.

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