result of increased release of vitamin A from the liver. Since the ester level is not significantly raised in this condition, the hypervitaminemia is apparently not, as it has been assumed,[5] due to reduced uptake of vitamin A by the liver during the recovery period when the absorption has returned to normal but liver damage still persists. The fact that the blood vitamin A alcohol represents vitamin A released from the liver explains also its reduction in malnutrition, infections, hepatic and wasting diseases, *i.e.* conditions in which the hepatic vitamin A concentration is reduced as in chronic liver diseases or malnutrition, or vitamin A release is impaired as in acute diseases (pneumonia or hepatitis). This impairment was previously considered morphologically the result of a displacement of vitamin A in the liver cells[2] and now is believed, physiologically the result of impaired esterification.[6]

The reduction of the vitamin A alcohol concentration under pathologic conditions, often associated with a rise in esters, elevates the ester percentage like the intake of vitamin A, but obviously on an entirely different basis.

Although the total plasma vitamin A level is shown to depend as much on endogenous[7] as on nutritional factors, it is often used as a valuable index of general or vitamin A nutrition. However, the vitamin A alcohol level is a superior index because it reflects much better storage in and release from the liver and is independent of solubility and postprandial rise. Despite the more elaborate method of determination, the vitamin A alcohol level may replace the total plasma vitamin A level as a nutritional index.

Summary. Plasma vitamin A esters rise markedly after intake of vitamin A. They are elevated significantly in nephrosis and slightly and irregularly in conditions associated with hepatic impairment. The plasma vitamin A alcohol level does not change after the intake of vitamin A but is elevated in nephritis and sometimes in recovery from conditions associated with hypovitaminemia A. It is significantly decreased in malnutrition, infections, hepatic and wasting diseases, apparently due to reduced storage in and/or release from the liver. The vitamin A alcohol level, therefore, excels the total vitamin A level as index of hepatic vitamin A storage and vitamin A nutrition.

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Probability of Normal Development after Transplantation of Fertilized Rabbit Ova Stored at Different Temperatures.

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In 1890 and 1897, Heape[1] reported his successful transplantation of rabbit ova to a uterine foster mother. This was mainly a demonstration of the unimportance of somatic tissue to the germ cells. Pincus and Enzmann[2] obtained normal young at term by transplantation of rabbit ova fertilized or cultured *in vitro*. In my last communication,[3] optimal temperature for the storage of ova and the normal development of young after low temperature storage of ova was reported. Since then, I have obtained the following data which show the probability of normal development.

Methods. The ova were obtained from

[1] Heape, W., *Proc. Roy. Soc.*, 1890, **48**, 457; 1897, **62**, 178.

[2] Pincus, G., and Enzmann, E. V., *Proc. Nat. Acad. Sc.*, 1934, **20**, 121.

[3] Chang, M. C., *J. Gen. Physiol.*, 1948, **31**, 385.

TABLE I.

Probability of Development Following Transplantation of Fertilized Rabbit Ova, Recovereed at Different Stages and Stored at Different Temperatures for Various Lengths of Time. F. Recovered from or transplanted into oviducts. U. Recovered from or transplanted into uterus.

Time of recovery after insem.	Storage or culture		Recipient does		Total ova trans.		Fate of ova in pregnant does							
	Time, hr	Temp., °C	No.	Preg., %	No.	Normal devel., %	No. of ova	Normal devel., %	Degen. before implantation, %	Degen. after implantation, %	Sex of young: Male	Female	Dead	Total
25 F.	1-2	30	24 F.	91.6	239	54.4	209	62.2	30.6	7.2	61	60	9	130
72 F.U.	"	"	11 U.	45.5	132	31.1	57	71.9	24.6	3.5	13	15	12‡ + 1	41
96 U.	"	"	9 U.	55.6	89	23.6	43	48.8	48.8	2.3	11	10	0	21
25 F.	"	"	8* U.	12.5	109	.9	13	7.7	92.3	0	0	1	0	1
25 F.	"	"	6† U.	0	92	0	0	0	100.0	0	0	0	0	0
25 F.	24	38	13 F.	61.5	137	20.4	75	37.3	42.7	20.0	16	10	2	28
25 F.	48	"	9 F.	66.7	153	26.8	101	40.6	47.5	11.9	19	17	5	41
25 F.	72	"	6 F.	0	109	0	0	0	100.0	?§	0	0	0	0
25 F.	24	10	7 F.	57.1	94	37.2	50	70.0	20.0	10	15	20	0	35
72 F.U.	24	"	7 U.	85.7	103	36.9	86	44.2	39.5	16.3	21	16	1	38
96 U.	24	"	8 U.	87.5	138	18.8	118	22.0	66.1	11.9	15	10	1‖	26
25 F.	96	"	14 F.	64.2	182	15.4	123	22.8	68.3	8.9	12	14	2	28
72 F.U.	96	"	4 U.	75.0	46	45.7	31	67.7	29.0	3.2	13	8	0	21
25 F	24	0	7 F.	42.9	91	11.0	35	28.6	71.4	?§	4	4	2‡	10
25 F.	96	"	9 F.	44.4	146	6.2	56	16.1	82.1	1.8§	1	8	0	9
72 F.U.	96	"	4 U.	0	59	0	0	0	100.0	?§	0	0	0	0

* Does ovulated 24 hr before operation.
† Does ovulated 72 hr before operation.
‡ Mother dead.
§ 1 or 2 animals diagnosed as pregnant at 2 weeks.
‖ Abnormal young.

superovulated rabbits[4] and transplanted within 1 to 2 hours or stored in undiluted rabbit serum at 0° C (cooled down in 5 hours) or at 10° C, or cultured at 38° C (subculture every 24 hours) for various lengths of time and then transplanted. The recipient does were ovulated by injection of gonadotrophin 1, 3, and 4 days before the operation according to the stage of ova recovered, irrespective of the time interval of storage or culture. Ova recovered from oviducts 25 hours after insemination (mostly in the 2 cell stage) were transplanted into oviducts through fimbria, while those recovered 72 hours (mostly in the morula stage, some in the oviducts and some in the uteri) and 96 hours after insemination (some in the blastocyst stage, nearly all in uteri) were transplanted into uteri by puncturing the uterus with a pipette containing ova. In 2 series of experiments, the ova at 2 cell stage were transplanted into the uteri of recipient does who had been ovulated 1 or 3 days before operation to determine receptibility of uterus to the ova at early stage. In each case 5 to 20 ova were transplanted depending on the time interval of storage, *i.e.*, more ova if stored for a longer time. Most of the pregnant recipient does were killed 25 to 28 days after operation. The number of normal young, the number of dead fetus in normal form (considered as normal development), the number of fetal placenta (absorption of embryo or fetus), and the number of maternal placenta (ova implanted but failing to develop) were counted.

Results. The complete results are presented in the accompanying table. The following facts are apparent from the data: (1) Of the total ova freshly transplanted, the percentage of normal development decreases from 54.4 to 23.6 when the ova were recovered from 25 to 96 hours after insemination. Thus, the younger the ova, the better the chance for normal development. (2) When the ova were cultured at 38° C for 24 or 48 hrs., the percentage of normal development is 20.4 or 26.8 respectively, whereas for 72 hours, no young were obtained. (3) For storage at 10°C for 24 hours, the percentage of development of the total ova transplanted is 37.2, 36.9 and 18.8, when the ova were recovered 25, 72, and 96 hours after insemination. Considering the percentages of development in the pregnant does (70, 44.2, and 22), it is again demonstrated that the younger the ova the better the chance for survival after storage. This percentage is 15.4 or 45.7 after storage at 10°C for 4 days with the ova recovered 25 or 72 hours after insemination. The latter percentage is exceptionally high, perhaps due to the small number of cases. (4) At 0° C, the percentage of normal development is very low even when stored for 24 hours (11%). But normal young were obtained after storage for 4 days at 0° C (6%). It seems, therefore, that 10° C is the optimal temperature for storage of ova as determined in a previous study on cleavage *in vitro*.[3] (5) It is interesting to note that even freshly recovered 2-cell ova cannot survive as embryos in the uterus regardless of whether the recipient does were ovulated 24 or 72 hours before transplantation. It seems that the 2-cell ova can survive *in vitro* at 0°, 10°, or 38° but fail *in utero*. Although the chance of survival *in vitro* is better in the case of ova recovered early, the results clearly show that only ova developed to a certain stage can survive and develop *in utero*.

The percentage of ova recovered, as checked by the number of ovulation points in the ovaries, was about 90% from oviducts. It was about 40% from the uterus 72 hours after insemination, but about 65% from uterus 96 hours after insemination. The sex ratio of the offspring was not altered after storage of ova *in vitro;* therefore, there is no obvious differential mortality of male or female zygotes at different temperatures *in vitro*. Only one abnormal young (hernia and deformation of fore limbs) was obtained in this investigation, but indications of degenerated embryos were observed in each treatment. It is highly probable that the defective ova or embryos were degenerated and absorbed rather than developed into abnormal young.

The percentage of ova degenerated at dif-

[4] Pincus, G., *Anat. Rec.*, 1940, **77**, 1.

ferent stages in the pregnant recipients as recorded in the table may be lower than what happened in all the recipient animals because all non-pregnant does were not sacrificed for examination. Several animals were determined as pregnant by palpation 2 weeks after operation but found to be not pregnant at a later stage, presumably due to the early degeneration of embryos. In general, the percentage of ova failing to implant is higher when the ova were recovered at a late stage and/or when they were stored or cultured. The percentage of degenerated embryos or fetus is higher after storage or culture of ova (8.9 to 20%) as compared with fresh transplantation (2.3 to 7.2%).

The large number of ova produced by gonadotrophin administration are capable of developing into normal young as is demonstrated in the following cases: In one case, 53 two cell ova recovered from one doe were freshly transplanted into 4 does and 45 normal young were obtained. In another case, 26 young were obtained from 3 recipients by the transplantation of 35 two cell ova from one doe.

Discussion. In view of the fact that, in the rabbit, about 33% of ova degenerate[5] in the course of development following natural mating, the effectiveness of transplantation of freshly recovered two cell ova (54.4 to 62.2%) is nearly similar to the natural course. While the transplantation of two cell ova after 24 hours at 10°C (37.2 to 70%) is about 15% lower, and that of morulae and blastocysts is about 30% lower than the natural course.

For the full utilization of the germ cells of valuable animals, there is a great difference between the spermatozoa which are produced by the thousands of millions in one ejaculation and the ova which are only one or two in number per heat period. However, considering the fact that one ovum requires millions of spermatozoa to insure fertilization and the fact that the number of ova ovulated can be much increased by the administration of gonadotrophins, it seems that there may be practical value in developing the technic of ovum transplantation and related fundamental research for animal industry.

Summary. The percentages of fertilized rabbit ova developed into normal young after transplantation of the ova recovered at different stages (from 2-cell ova to blastocysts), and stored at different temperatures (0°, 10°, 38° C) for various length of time (24 to 96 hours) into the oviducts or uteri of recipient does were reported.

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[5] Hammond, J., *Z. f. Pelztier- und Rauchwarenkunde,* 1931, **3**, 56.