ficant change in the lymphocytes (16% decrease in Case 8 and 39% increase in Case 9).

We have found no reports on eosinopenia following insulin hypoglycemia in either psychotics or normal individuals. Godlowski(13) demonstrated, however, a similar significant decrease in eosinophiles of eleven allergic patients with eosinophilia after the subcutaneous administration of 100 units of insulin. The period during which maximal eosinopenia occurred was 6 to 8 hours after the administration of insulin in his series. This is comparable to our results of 4 to 8 hours after intramuscular administration of insulin.

Lymphopenia following insulin hypoglycemia in psychotics has been observed by Katzenelbogen(14) and Parsons(15). The period during which maximal lymphocytopenia occurred in Parsons' series was 4 to 6 hours after intramuscular insulin administration, which is identical with our findings.

As mentioned above, insulin hypoglycemia causes an outpouring of epinephrine(3,4), which stimulates the production of ACTH by the anterior pituitary(6,7). It is probable therefore that the adrenocortical activation in insulin hypoglycemia depends on the liberation of epinephrine from the adrenal medulla. The quantitative and qualitative similarity of the falls of eosinophiles following insulin and epinephrine in allergic subjects, as observed by Godlowski(13), and the same similarity of the falls of lymphocytes following insulin and epinephrine in psychotics, as observed by Parsons(15), support this view. The delayed occurrence of eosinopenia and lymphocytopenia following insulin as compared with epinephrine and electric shock(13, 15) can be explained by the time interval required for the hypoglycemic effect of insulin and the subsequent output of epinephrine from the adrenal medulla to take place. When hypoglycemia was prevented by glucose (Cases 7 and 8) no significant eosinopenia and lymphocytopenia occurred. This also supports our belief that epinephrine is the cause of adrenocortical activation in insulin hypoglycemia.

Further studies, including the concurrent employment of adrenolytic drugs during insulin hypoglycemia, are in progress.

Summary. Insulin hypoglycemia causes in schizophrenics a significant eosinopenia and lymphocytopenia, which is considered to be indicative of adrenocortical stimulation. Insulin does not produce such changes if hypoglycemia is prevented.

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15. Parsons, E. H., Gildea, E. F., Ronzon, E., and Herbeit, S. Z., *Am. J. Psychiatry*, 1949, v105, 573.

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Culture of Brown-Pearce Carcinoma in the Embryonated Egg. (18049)

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The Brown-Pearce carcinoma is commonly maintained by rabbit inoculation. Since the tumor may regress or die out during the summer months, and because of obvious economic and experimental advantages, we have investigated the feasibility of maintaining the growth in the developing egg. The present experiments show that the Brown-Pearce carcinoma may be cultured readily on the chorioallantoic membrane of the chick, and that it retains its capacity for growth and metastasis in the rabbit. Data are also presented on some conditions conducive to an optimal number of "takes" on the chorioallantoic membrane.

Materials and methods. Brown-Pearce tissue was obtained from female Dutch rabbits inoculated by the intra-uterine technic of

TABLE I.
Growth of Brown-Pearce Implants on Chorioallantois of the Chick Embryo.

Subculture	Total eggs	No. embryos surviving	No. surviving embryos with takes	% takes in surviving embryos
		Experiment A		
1	24	19	12	63.2
2	10	9	8	89.0
3	8	8	8	100.0
4	15	13	10	77.0
5	19	17	13	76.5
6	10	7	5	71.4
Totals	86	73	56	76.4
		Experiment B		
1	12	8	5	62.5
2	5	5	2	40.0
3	3	3	2	66.0
4	6	6	3	50.0
5	7	7	7	100.0
6	11	10	8	80.0
7	11	9	5	55.6
8	9	7	4	57.1
Totals	64	55	36	65.5
		Experiment C		
1	35	28	16	57.3
2	11	10	6	60.0
3	5	5	2	40.0
4	5	5	3	60.0
5	5	5	5	100.0
6	5	3	1	33.0
7	3	2	2	66.0
Totals	69	59	35	59.3
Grand totals	220	187	127	67.9
		*Experiment D**		
1	145	102	99	97.0

* Tumor tissue maintained *in vitro* in rabbit serum for 2 to 6 hr before implantation on the chorioallantois.

Dr. Elly M. Jacobsen.* Fourteen days after inoculation of the rabbits the metastasized tissue was removed aseptically and transplanted onto the chorioallantois of New Hampshire Red eggs which had been incubated for 8 days at 37.5°C. The chorioallantoic method described by Rugh(1) was used with the exception that the window in the shell was covered with 2 layers of sterile cellophane cut out of flat dialysis tubing and fastened to the shell with 1/4 inch wide strips of scotch tape. Pieces of tumor of approximately 1 mm cube were placed upon a large chorioallantoic vessel, and the sealed eggs were then maintained in the horizontal position without rotation for about 9 days. At this time the tumor mass, together with a portion of the adjacent membrane, was removed to a sterile saline-moistened paper towel in a watch glass. For subculture bits of tissue were immediately transferred to the chorioallantoic of new 8-day embryos. In several series the attached membranes were removed, excess fluid drained off with filter paper, and the wet weights obtained. Thirty-eight tumors were fixed in Bouin's fluid, sectioned at 8 μ and stained with Delafield's hematoxylin and eosin, and smears were made of blood from vessels serving the site of the tumor. The egg-cultured tumors were finally re-implanted into the rabbit *via* the anterior chamber of the eye in order to ascertain

* We are indebted to Dr. Jacobsen for providing us with tumor tissue and for generous aid and counsel.

1. Rugh, R., *Exp. Embryol.*, 1948, 441.

TABLE II.
Results Obtained with Single and Multiple Implants of Brown-Pearce Tissue on Chorioallantois of the Chick.

	No. surviving embryos	No. surviving embryos with takes*	% surviving embryos with takes
Single implants	70	41	58.5
Multiple implants	113	85	75.2

* The term "take" refers to the presence of one or more growths per egg.

possible alterations in the virulence of the growth.

Percentages of successful grafts. Table I summarizes the results of 3 experiments (A, B, C), each initiated with tumor tissue from a separate rabbit. The tissue was allowed to grow in the chick for 6-10 days (most commonly 9 days) and then grafted into a new group of eggs. The percentage of successful "takes" varies from 33% to 100% in various subcultures, with an average of 67.9% for embryos surviving to the 17th day of incubation. On the basis of the total number of eggs implanted 57.7% show successful grafts on the 17th day. It will be noted that there is apparently no trend toward increase or decrease of successful takes in successive subcultures.

In experiments A, B, C (Table I), the tumor tissue was placed upon saline-moistened paper towelling and portions were then immediately transplanted. In a second series of experiments, the excised tissue was separated into 1 mm cube fragments, incubated in rabbit serum for 2-6 hours at 37°C, and then transplanted as usual onto the chorioallantois. As indicated in Table I (Experiment D), the percentage of successful grafts was significantly increased, although we are unable to conclude that the superiority of this method is due to the serum rather than to the period of maintenance *in vitro*, incubation at 37°C, or to a combination of these factors.

The percentages of successful takes in eggs receiving only one tumor implant as compared with others receiving 2-4 implants per egg, are given in Table II. The apparent superiority of multiple grafts may possibly be due to injury of the tumor tissue during the course of subculture; however, observation suggests that implants frequently are displaced, whether by embryonic movements or other factors, from the original site of implantation. It may be suggested that the presence of several grafts makes it more likely that at least one of them will retain its position upon a large blood vessel.

Weight increase. In 3 series of grafts the growths were removed from the membrane after 8-9 days of culture, blotted with filter paper and weighed. On the basis of the tumors obtained from 37 eggs the wet weight of the tumor shows an average increase of 79 times over the weight of the original implant. Actually, the average growth rate must be far greater than is indicated by these values, since the active tumor mass originates from a small portion of the implanted tissue (Fig. 2). An indeterminate but certainly greater portion of the implant remains on the chorioallantois as a necrotic mass.

Re-implantation into rabbits. Tumors were removed from the chorioallantois at various subcultures and re-implanted into the anterior chamber of the rabbit eye. As shown in Table III, the egg-cultured tumors grew in every instance, irrespective of the age of the host, and there was definite metastasis in 8 of the 14 rabbits used. It is of interest to note that the adrenals were involved in 7 of the 8 animals showing metastases, whereas the mesenteries and serosa were affected in only one instance and the liver was entirely free of macroscopic growths.

Histology. The tumor *in situ* usually has the appearance of a pinkish mass adherent to the median surface of the chorioallantois. Smaller growths commonly show a thick whitish peripheral zone and a dark red central core; larger growths (approximately 10 x 10 x 10 mm) are almost entirely dark red, the light peripheral zone being extremely thin. The large vessels of the chorioallantois may

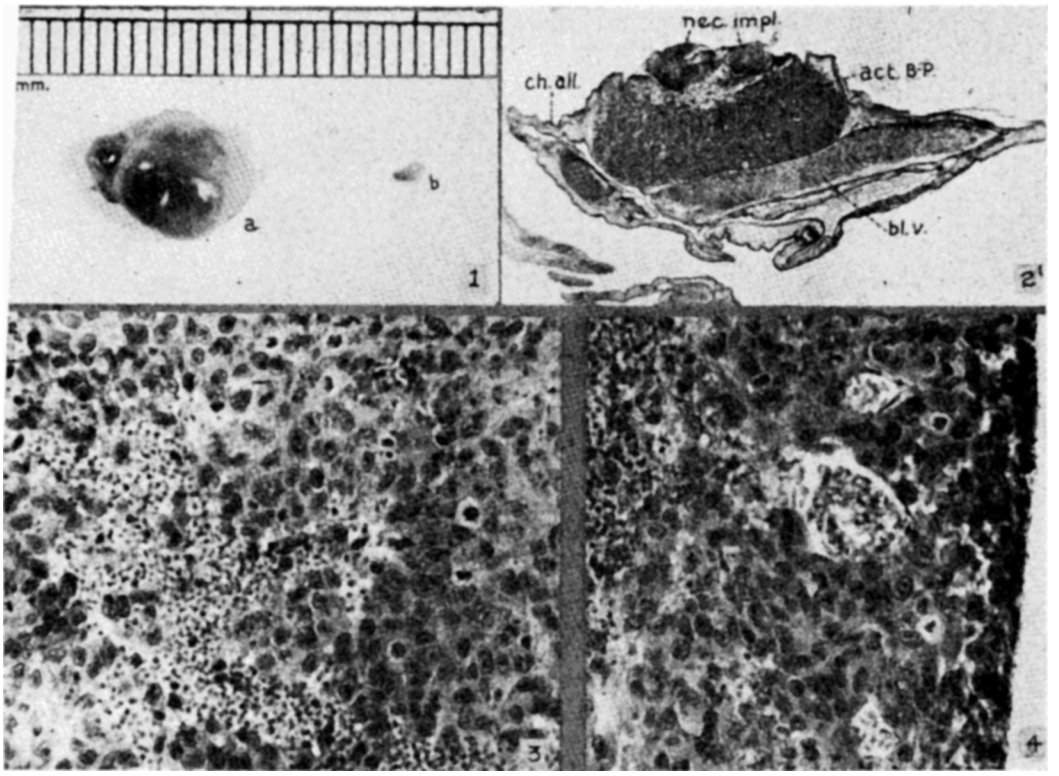

FIG. 1. Typical large tumor (a) cultured on the chorioallantois for comparison with piece of tumor used as implant (b).

FIG. 2. Section through a tumor of moderate size cultured on the chorioallantois. *nec. impl.*, necrotic implants. *act. B-P.*, active Brown-Pearce cells. *bl. v.*, chorioallantoic blood vessel. *ch. all.*, chorioallantois.

FIG. 3. Section showing the central region of a tumor grown on the chorioallantois. The large Brown-Pearce cells are seen in intimate contact with embryonic blood, without intervening endothelium.

FIG. 4. Section of a tumor showing a peripheral portion with endothelial cells visible and Brown-Pearce cells packed about the vessels.

be seen on the median aspect of the tumor (Fig. 2), indicating that the growth pushes the vessels before it toward the interior of the egg. The original implant is discernible as a dark brown or almost black necrotic mass imbedded in the membrane immediately above the actively growing tumor tissue (Fig. 2).

Thirty-eight tumors representing specimens taken from various subcultures through the 8th, present a quite uniform histological picture. The tumor cells proliferate locally in the mesodermal stroma of the membrane, deflecting the endoderm and large blood vessels (Fig. 2). In contrast with growths of similar size in the rabbit, even the larger tumors show little or no connective tissue stroma among the tumor cells. In further contrast with the larger tumor nodules in the rabbit, growths of corresponding size in the chick show no necrotic areas, the entire growth being composed of active Brown-Pearce cells as far as may be judged from histological characteristics (Fig. 3, 4). In the central region of the growth the tumor cells are intimately mingled with chick blood cells present in spaces which apparently lack an endothelial boundary (Fig. 3). In peripheral regions of the tumor, vessels lined with endothelium are closely packed with Brown-Pearce cells, but in no instance were tumor cells observed within the vessel (Fig. 4).

In view of the intimate contact of the

TABLE III.
Implantation of Brown-Pearce Tumor Grown on Chick Chorioallantois into the Anterior Chamber of the Normal Rabbit Eye.*

Animal No.	Rabbit age (mo.)	Source of tissue† Exp.	Subcult.	Growth in anterior chamber¶	Period of growth in anterior chamber (wk)	Metastases
122‡	unknown	C	1	Yes	12	Possibly right adrenal
124	18	C	1	"	10	Nodules on serosa of gut, mesenteries, peritoneum and omentum
136	15	B	3	"	11	None
139	16	B	3	"	8	"
140§	12	B	1	"	6	Nodules on kidney, right and left adrenals
144	15	C	2	"	11	None
1	4.5	A	2	"	10	Right adrenal, right eye
2	4.5	A	2	"	10	" " " "
3	4.5	A	2	"	10	Right and left kidneys, right adrenal
4	4.5	A	2	"	10	None
5	5	B	5	"	9	Rt. kidney, rt. adrenal
6	5	B	5	"	9	None
7	5	B	5	"	9	Right adrenal
8	5.4	C	4	"	9	Left "

* Unless otherwise indicated, implantation was made into the left eye.
† Tumor tissue taken from the experiments and subcultures indicated in Table I.
‡ Implantation into right eye.
§ " " both eyes.
¶ Growths in all instances completely filled the anterior chamber; in many the entire eye was replaced.

tumor cells with the embryo's blood, especially in the central region, it is surprising that no metastases could be found, although most of the chicks were autopsied with this purpose in mind. Furthermore, histological examination of the embryonic liver, spleen, gall bladder, mesonephros, heart, and brain in a number of specimens gave no evidence of metastases nor of any pathological change. Smears made of blood from vessels immediately supplying and draining the tumor site showed no Brown-Pearce cells in any instance. The only observed effects of the tumor on the chick are limited to the localized region of the chorioallantois overlying or surrounding the growth. Here the membrane is translucent and slightly wrinkled, and may show ectodermal proliferation and apparent keratinization as already described by Campbell (2). The chick proper shows no apparent effect of the tumor and a number of specimens allowed to hatch, observed for several weeks, and then autopsied were quite normal.

The failure of the tumor to metastasize in the chick may depend upon an antagonistic factor present in the chorioallantois and is worthy of further study. The anti-metastatic effect does not persist after removal of the tumor from the chick, since metastasis occurs upon re-implantation into the rabbit. Lee *et al.*(3) obtained metastases of mouse tumor by injecting the tumor into the allantoic vein, which suggests that the vascular wall possibly acts as a barrier. However, this seems a highly improbable explanation insofar as the Brown-Pearce tumor is concerned, since the tumor cells appear to be in direct contact with the embryonic blood in the central region of the growth.

Summary. 1. The Brown-Pearce carcinoma of rabbits shows definite growth on the chorioallantois in 58% of embryos implanted on the 8th day of incubation, and in 68% of embryos surviving until the 17th day. The wet weight of the tumor increases 79 fold

2. Campbell, J. G., *Brit. J. Cancer*, 1949, v3, 72.

3. Lee, H. F., Bender, D. H., and Friedgood, C. E., *Science*, 1948, v107, 374.

during the 9-day culture period. 2. The average percentage of successful grafts is increased by implantation two or more pieces of tumor within a single egg, and by incubating the excised tumor for several hours in rabbit serum before implantation onto the chorioallantois. 3. The tumor does not metastasize from the chorioallantois in spite of apparent direct contact between the embryonic blood and the tumor cells.

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Use of Antipyrine in Measurement of Total Body Water in Animals.* (18050)

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A method for determining total body water in man by means of antipyrine has recently been described(1). In a series of tests this drug fulfilled the characteristics a substance should possess for the measurement of total body water in man: It was not toxic in the amounts necessary for the determination, it was distributed evenly and rapidly throughout the body water, it was degraded and excreted at a regular rate, and the analytical procedure for its determination was accurate and simple.

Most methods for measuring total body water are based on the volume of distribution of a foreign substance after intravenous administration. These procedures are in the main unsatisfactory because of unequal distribution in the tissue water of the body. In contrast, determining total body water with deuterium oxide or tritium is more satisfactory as these isotopes distribute evenly, but the analytical technics are difficult and the cost high(2,3,4).

Antipyrine is rapidly transformed in the dog and it was thought that this characteristic would preclude its use for the measurement of total body water. The following studies were carried out to ascertain whether or not antipyrine is in fact satisfactory for this purpose.

Methods. Measurement of antipyrine in tissues and plasma has been described‡(5). Following intravenous injection, the distribution of antipyrine was determined in representative tissues of one rabbit at 50 minutes following injection and 2 dogs at 1½ hours and 2¾ hours respectively. The animals were killed by intravenous air injection and the tissues were analyzed immediately.

The following technic was employed to measure total body water: control sample of blood was withdrawn to determine the plasma blank value for correcting subsequent samples; 75 mg of antipyrine per kilo body weight in the form of a 5% solution in distilled water was injected intravenously from a burette or calibrated syringe; blood samples were withdrawn and heparinized at 2, 3, and 4 hours after injection; plasma and cells were

* The technical assistance of James J. Dooley and Mrs. Mary M. McLaughlin is gratefully acknowledged.

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1. Soberman, R., Brodie, B., Levy, B., Axelrod, J., Hollander, V., and Steele, J. M., *J. Biol. Chem.*, 1949, v179, 31.

2. Pace, N., Kline, L., Schachman, H. K., and Harfenist, M., *J. Biol. Chem.*, 1947, v168, 459.

3. Hevesy, G., and Hofer, E., *Nature*, 1934, v134, 879.

4. Moore, F. D., *Science*, 1946, v104, 157.

‡ The method is based on the addition of sodium nitrite to a plasma filtrate or tissue extract with the resulting formation of a 4-nitroso antipyrine which can be read in the spectrophotometer at 350 mu. Appropriate dilutions of the plasma are made so that readings fall in the significant colorimeter range.

5. Brodie, B., Axelrod, J., Soberman, R., and Levy, B., *J. Biol. Chem.*, 1949, v179, 25.