result suggested an insufficient sensitizing dose and too short incubation time.

Twelve guinea pigs were sensitized by the method previously described. The sensitizing dose of pneumococcus culture was equal to the amount of organisms grown upon 2 blood agar slants in 24 hours' incubation. Four weeks later the anaphylactic test was performed and the result presented in Table I was obtained.

TABLE I.
Showing Active Sensitization of Guinea Pigs with Pneumococcus Type I Cultures.

No. of animals	Dose of specific carbohydrate	Results
	mg.	
2	2	No response
3	4	Dead in 3-5 minutes
2	4	Moderate shock
3	6	Very slight shock
2	8	No response

From this table it follows that altogether 5 animals developed typical anaphylaxis. Three of these animals died in 3-5 minutes while 2, after having developed a moderate anaphylactic shock, recovered. The autopsies performed on the dead animals revealed typical distension of the lungs. As seen from the table, the effective dose of the carbohydrate was found to be equal to 4 mg. Doses smaller or larger than this produced either very slight or no effect. Our experiment suggests that sensitization of guinea pigs with heat-killed cultures of pneumococcus type I requires comparatively large doses of the microörganism and proper adjustment of the dose of the specific carbohydrate.

9020 C

Growth of Cancerous and of Embryonic Tissues Stratified in the Ultra-Centrifuge.*

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In an attempt to upset the normal conditions of cells and thus possibly induce abnormal growths which might give some insight into malignancy the authors subjected various bits of embryonic

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tissues to intense centrifugalization and implanted them in young rats of 65-90 gm. weight. Small pieces of rat carcinoma were similarly centrifuged and implanted. A Beams air-driven ultracentrifuge, rotated at a speed which produced a displacement pull of about 400,000 times that of gravity, was employed. The embryonic tissue consisted of small bits snipped from the body-wall of living embryos about 2 weeks old. It was placed immediately in isotonic Locke's solution. Half of each bit was transferred to the metal rotor of the centrifuge and the other half implanted subcutaneously into a control rat. Microscopical inspection showed that the cell-contents of the various tissues used had been stratified in the centrifuge in practically the same way as that previously reported for pituitary gland.[1] Representative results which followed implantation are shown in the following paragraphs.

Cancer. In a preliminary test, cancer tissue (Flexner Jobling carcinoma) centrifuged for 10 minutes was implanted in 2 young rats, and similar cancer tissue centrifuged for 20 minutes was implanted in 2 other young rats. Inasmuch as the tissue centrifuged for 10 minutes grew week by week, during 9 weeks of observation, at practically the same rate as non-centrifuged control implants, it was evident that such tissue could withstand the treatment unimpaired. The cancer tissue centrifuged for 20 minutes did not start to grow in one of the host rats; in the other it grew slowly for 6 weeks then regressed from a diameter of 9 mm. to 5 mm. during the next 3 weeks.

As a result of these exploratory tests it was decided to try centrifugalization for about 15 minutes with a sufficiently large number of transplants to give significant results. Accordingly bits of carcinoma measuring about 1 mm. in diameter were centrifuged for 15 minutes and then implanted into each of 10 young rats weighing from 70-90 gm. Of the 10 implants one did not "take". The remaining 9 were observed and measured week by week. All started active growth but by the end of 5 weeks 2 were regressing and the other seven were growing at a rate indistinguishable from that of the controls.

Since 15 minutes of centrifuging produced no perceptible change in the implants, a second series of transplants was made, using cancer tissue centrifuged for 20 minutes. Such centrifuged tissue together with corresponding bits which had been kept in Locke's solution for the same length of time were implanted in 10 young rats (65-90 gm.), a centrifuged piece and a non-centrifuged con-

[1] Guyer, M. F., and Claus, P. E., *Biol. Bull.*, 1936, in press.

trol in each rat. The transplants grew in each of the 10 hosts. In one individual both the centrifuged and the non-centrifuged cancerous growths began to diminish in size after the ninth week and 4 weeks later both areas were healed and the rat free of all cancerous tissue. In the 9 other individuals the growth of each implant was continuous with no significant differences between the experimental and the control tumor, hence the experiment was terminated at the end of 12 weeks.

It is of interest to note that the carcinomatous tissue in question showed less indication of stratification than any tissue we have subjected to prolonged centrifugalization. After 20-30 minutes of such treatment the nucleus though commonly showing some evidence of stratification within itself, underwent little or no dislocation in the cytoplasm, and the cytoplasm seemed little disturbed. Even the Golgi apparatus which exists in a more or less diffuse condition in such cancer cells, largely retained its ordinary distribution. Such evidence would seem to indicate that the cytoplasm of the carcinoma cells in question was physically in the condition of a very stiff gel.

Trophoblast. Trophoblastic tissue removed during early pregnancy and centrifuged for 12 minutes did not grow when implanted into each of 5 young rats. However, similar tissue, not centrifuged, likewise did not grow when implanted into 4 control rats, hence the lack of viability can not be attributed to the centrifugalization.

Body-wall. Small pieces about 2 mm. in diameter, snipped from the body-wall of rat embryos between 10 days and 2 weeks old, were subjected to centrifugalization. The thought behind the experiment was that active mitosis would be in progress and that, therefore, as a result of centrifugal displacement irregular cell divisions might be induced and thus lead to abnormal tissue developments. Similar pieces of tissue were kept in Locke's solution for the same length of time the centrifuged bits were out of the body; they were then implanted as controls in young rats of the same age as those implanted with the centrifuged tissue.

In the first experiment 10 young rats (65-90 gm. in weight) were implanted subcutaneously with bits of embryonic body-wall which had been centrifuged for 12 minutes, and 6 controls were implanted with similar bits of uncentrifuged tissue. Microscopical inspection showed that the centrifuged cells had been much flattened and stratification of cell contents was evident, with the nucleus displaced to the centrifugal side of the cell. The centrifuged tissue resumed growth in 9 of the 10 hosts to which it was transferred, and the non-centrifuged in 5 of the 6 controls. The growth, indicated ex-

ternally by elevations of the skin, was observed and measured week by week. The tissue was removed at the end of 12 weeks from 3 of the hosts, and at the end of 32 weeks from 5 others, and sectioned for study. In one of the 9 animals in which the centrifuged tissue grew, growth ceased at the end of 4 weeks and by the end of 8 weeks the transplant had wholly disappeared. In the other 8 growth continued and reached its maximum in about 8 weeks after which the transplants maintained themselves at the maximal size until removed for examination. The subcutaneous masses, measuring 1.7x1.2, 1.5x1.0, and 1.2x0.7 cm. respectively, removed at the end of 12 weeks proved to be rounded capsules filled with hair. Similarly 2 of the subcutaneous growth masses removed at the end of 32 weeks, measuring 0.5x0.5 and 1.8x1.2 cm. respectively, contained capsules filled with hair. Evidently, although neither hair nor well developed skin were present in the small bit of implanted tissue, less than 2 mm. in diameter, these structures were already determined *in potentia* and despite the violent treatments in the centrifuge the tissue continued to increase in mass and eventually developed into skin bearing hair follicles and hair that seemed in no wise different from that of normal rat skin, except the hair was somewhat longer. That the centrifuging had nothing to do with the phenomenon is evident from the fact that similar capsules containing skin and hair masses developed in 3 of the controls. In one of the rats bearing a centrifuged bit of embryonic body-wall, at the end of 32 weeks the transplant had developed into a flat piece of cartilage and bone measuring 1.3x0.7 cm. Histologically (Fig. 1) the tissue did not look different from that of an ordinary rib undergoing ossification although it was much larger than any normal rib of its host. Presumably a bit of what would have eventually been rib cartilage or bone was present in the centrifuged material and it continued its inherent course of development. The obviously abnormal thing about it was its large size, due probably to a lack of the mechanical and other restraining factors that prevail when such a tissue grows in its normal position as a rib.

In a later experiment a similar growth of cartilage and bone developed from a centrifuged bit of body-wall. In this experiment 5 host rats were used and the bits of tissue from the body-wall were centrifuged 20 minutes before subcutaneous implantation. The rate and manner of growth of this transplanted tissue did not differ noticeably from that of the earlier experiment in which the tissues were centrifuged for 12 minutes. In 2 of the 5 hosts the transplants persisted for about 12 weeks and then disappeared. In 2 of the others encapsulated growths filled with hair were found when the

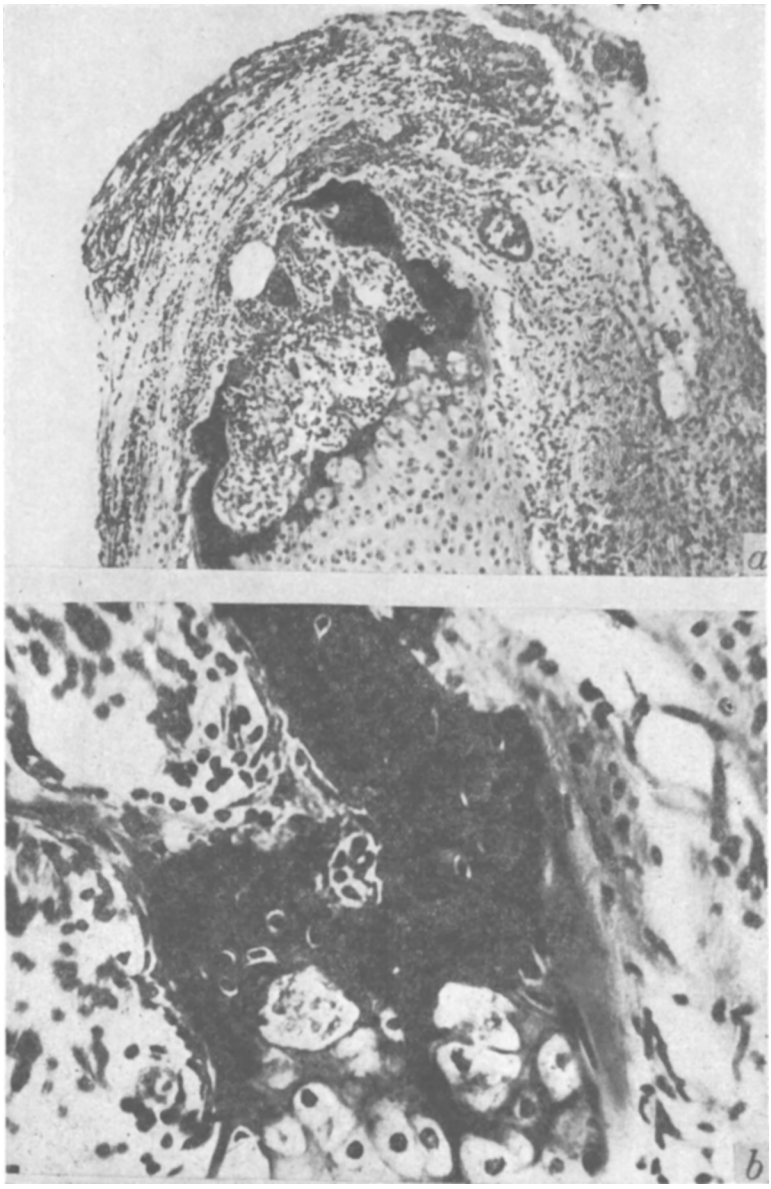

FIG. 1.

Section through part of a mass of cartilage and bone which developed in a rat from a bit of centrifuged embryonic body-wall: $a \times 100$; $b \times 970$. Ossified areas appear black; the cartilage cells are obvious.

transplants were removed at the end of 12 weeks. In the remaining host the centrifuged bit, as already stated, had grown into a flat piece of cartilage and bone which measured 1.5 by 0.7 cm.

The most significant result observed from the foregoing experiments was the persistence with which cells distorted by violent centrifugalization regained and maintained their usual characteristics. The abnormalities which appeared are probably interpretable as normal tissues developing in unusual locations rather than as the result of fundamental changes induced in the constituent cells by centrifugalization. The host seemed to be merely a nutritional matrix for the centrifuged tissue which developed along the path of its original constitutional trend.

Summary. The cells of carcinoma tissue, after 30 minutes of centrifugalization in an ultracentrifuge at a displacement pull of 400,000 times that of gravity, show little trace of stratification of contents. Apparently the cytoplasm of such cells is of the consistency of a stiff gel. Such cells centrifuged for 20 minutes grew as readily as non-centrifuged cancer cells when implanted in young rats. The cells in bits of embryonic body-wall centrifuged for 12 and for 20 minutes respectively, although having their contents markedly stratified by the treatment, resumed growth when implanted subcutaneously in young rats, developing usually into hair-filled cysts of skin but occasionally into cartilage and bone. These results were probably due to the misplacement of embryonic tissues rather than to changes induced by centrifugalization.

9021 C

Retarded and Prolonged Action of Insulin Precipitated by Safranin.

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The injection into man and animals of a suspension of insulin precipitated by a protamine has been shown to lower the blood sugar for a period several times as long as that given by ordinary insulin.[1] The appearance of an article by Walker[2] on the use of dyes to pre-

[1] Hagedorn, H. C., Jensen, B. N., Krarup, N. B., and Wodstrup, I., *J. Am. Med. Assn.*, 1936, **106**, 177; Root, H. F., White, P., Marble, A., and Stotz, E. H., *J. Am. Med. Assn.*, 1936, **106**, 180.

[2] Walker, A. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 726.