lobule-alveolar development appeared as it did in thyroidectomized rats similarly treated. It is possible that the period of thiouracil treatment was not continued for sufficient time for the changes to occur although thyroid removal for a similar period (35 days) did bring about these changes.[7] A lengthened estrous cycle was also produced by thiouracil, similar to that found in thyroidectomized rats.[10]

Estrogen injections into castrated rats will in time produce lobule-alveolar development but not in the 10-day periods of these experiments.[11] The hypothyroid state apparently causes a precocious appearance of the lobules and alveoli in the mammary glands under these conditions. The injections of thyroxin to offset the effects of thiouracil in inducing hypothyroidism were manifested in the failure of lobule-alveolar growth to appear in the mammary glands. Whether hypothyroidism brings about these changes in the mammary glands by altered hormone metabolism or by altered tissue responses is still a matter of conjecture.

Conclusion. Thiouracil administered to normal or castrated male and female rats failed to induce an alteration of the mammary glands similar to that obtained following thyroidectomy. The mammary glands of rats treated with thiouracil and with estrogen for the last 10 days of the experimental period possessed well developed lobules and alveoli whereas the control glands showed end bud growth and duct extension. When thyroxin was given along with thiouracil and estrogen, the mammary glands resembled those from rats receiving estrogen alone and no precocious lobule-alveolar growth was noted.

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The Use of Regenerating Liver as a Method of Assay.*

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Although the rate of mitosis in regenerating liver has been studied,[1] the procedure used was so laborious and time-consuming as to preclude its use as a method for testing the effect of agents upon the growth of liver cells. By applying the technic for obtaining free nuclei previously described by one of us,[2] a method of assay has been developed by means of which it has been possible to test as many as 10 substances in a week. The results of these assays will be published elsewhere.[3] We wish here to present results of studies of the relation of the age of the animal to the rate of mitosis and the uptake of P^{32} by the regenerating liver.

Method. An incision was made on the midline extending about ¼ to ½ inch on either side of the xiphoid process in an anteroposterior direction. The abdominal wall was cut along the midline for ¼ inch posterior to the tip of the xiphoid process which was dissected free of its attached muscle and fascia. With gentle pressure on the sides of the animal the median and left lateral lobes were delivered to the surface and their fascial attachments cut. They were then tied off at the base with cotton or linen thread and cut off as close as possible to the tie. To minimize

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TABLE I.
Liver 3 Hours After Intravenous P^{32}.

Day post-op.	% mitosis						P^{32}/g liver as % injected dose		
	n	1 mo	n	4-6 mo	n	16 mo	1 mo	4-6 mo	16 mo
0 *s* hep.	5	.07	6	.00	4	.01	3.16	1.78	1.78
0 *c* hep	3	.07	6	.03	4	.07	6.08	2.77	2.69
1	17	.74	8	.37	4	.25	5.52	2.81	2.19
2	5	.23	7	.47	4	.51	5.52	2.41	2.40
3	4	.20	6	.27	4	.98	4.86	2.25	2.43
4	3	.12	2	.18	4	.39	4.86	1.87	2.25
5	3	.06	4	.05	4	.13	3.51	1.88	2.14

n = No. of animals.

TABLE II.
Liver 24 Hours After Subcutaneous P^{32}.

	Days post-operative					
	1	2	3	4	5	6
% P^{32}/g liver	2.18	2.11	1.87	1.77	1.90	1.93
m	.047	.066	.054	.100	.115	.095
n	15	10	12	6	6	5
% Mitosis	.73	.28	.21	.07	.08	—

m = standard error of the mean.
n = No. of animals.

infection aseptic precautions were used throughout the operation. On a diet of Purina dog chow survival was 100% unless there was a gross error in the technic, *e.g.*, perforation of the diaphragm or cutting a large blood vessel. When the animals were put on a prepared diet containing 10 to 13% fat before the operation, survival was only 30 to 40%.

Twenty-four hours after the partial hepatectomy the animals were given P^{32} as Na_2HPO_4 intravenously via the femoral vein in doses not exceeding 1 mg Na_2HPO_4, 1.5 μc P^{32}/g of body weight. Three hours later the liver was perfused and removed for assay. In another set of experiments the P^{32} was given subcutaneously immediately after the operation and the liver was removed 27 hours later. A small portion of the liver was accurately weighed and its P^{32} content determined with a Geiger-Muller counter. For simplicity only nuclei in metaphase and anaphase were counted as being in mitosis. In all cases, the liver was removed between 1:30 and 4:00 p.m.

Rats of a hooded strain inbred by brother-sister mating in this laboratory for 10 generations were used in these experiments. Purina dog chow was fed *ad libitum*. The animals 1 month old weighed from 45 to 55 g, those from 4 to 6 months old weighed from 170 to 215 g, and those 16 months old weighed from 240 to 320 g. The animals in each group were given the same dose per gram of body weight on the basis of the average weight for each group, 50, 200, and 250 g respectively. No attempt was made to adjust the dose administered to the body weight within any one group. In calculating the percentage of P^{32} per gram of liver, the data were corrected to the average weight of the group, *e.g.*, to 50 g in the first group by multiplying by the observed weight and dividing by 50. From experiments to be reported subsequently, data are available for testing the validity of this correction. In 19 experiments, including 84 rats 1 month old, the weight ranged from 30 to 60 g, with a large majority between 45 and 55 g, and the mean percentage uptake of P^{32} 27 hours after operation and 4 hours after the injection of P^{32} was 4.517 $\pm$ .084 for the corrected and 4.728 $\pm$ .120 for the uncorrected. The variance ratio was 2.01 and P = 0.1 — 1.0%, that is, the variance is significantly greater in the uncorrected data.

The regression coefficients of the percentage of P^{32} on weight and their standard errors were calculated for the first 15 experiments (61 rats), since in the last 4 experiments there was no significant variation in weight. These were —0.09 ± 0.02 before and —0.008 ± 0.02 after correction, the effect of weight thus being almost entirely eliminated by the correction.

In Table I are shown the percentage of nuclei in mitosis and the P^{32} per gram of perfused liver at from 4 hours to 5 days after partial hepatectomy. Mitosis reached a maximum on the first, second, and third days for animals 1, 5, and 16 months old respectively. P^{32} was at a maximum at 0, 1, and 2 days for the same groups. There was an apparent correlation between the time required for reaching maximum mitosis and that for reaching the maximum uptake of P^{32}, the latter anticipating mitosis by 1 day. There was no significant change in mitotic rate during the first 4 hours after the operation.

In another set of experiments with rats weighing 50 g the P^{32} was given subcutaneously 24 hours before the liver was removed for assay. These results are shown in Table II. Maximum mitosis and uptake of P^{32} were reached at 1 day. Whether the P^{32} was administered intravenously or subcutaneously, the uptake of the radioactive element was a much less sensitive index of mitotic function of the liver than was the mitotic count itself. For example, while the uptake of P^{32} varied only a few percent between 0 and 1 a day, the mitotic rate increased by more than 1000%.

In animals which received P^{32} intravenously 1 hour after the partial hepatectomy, a greater concentration of P^{32} was found in the liver than in non-hepatectomized animals, which is probably due simply to the decrease in liver volume. This suggests that the progressive decrease of P^{32} found in the liver between the first and fifth days may be more directly related to the increase in bulk of the liver rather than changes in mitotic activity. However, the data cannot be considered to prove that P^{32} uptake is determined exclusively either by changes in liver volume or rate of mitosis.

Conclusions. 1. The time at which mitosis in regenerating liver reaches a maximum varies with the age of the animal. 2. P^{32} uptake by regenerating liver, 3 hours after intravenous administration of labelled Na_2HPO_4, varies with time after partial hepatectomy. This variation may be related to changes in liver volume or to mitotic activity or both. 3. P^{32} retention 27 hours after subcutaneous administration of labelled Na_2HPO_4, shows little variation with time after operation. 4. The mitotic rate provides a useful index which may be used in assaying the effect of various agents on the reproduction of liver cells.

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On the Use of Sodium Sulfadiazine in Surgery on Amphibian Embryos.

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One of the greatest difficulties encountered in surgery on amphibian embryos (especially Amblystoma) has been the unpredictable mortality. Particularly in experiments upon the central nervous system, if aseptic procedures are not followed, the death rate may be discouragingly high. Long experience has shown that complete superficial healing, following surgical interruption of the central nervous system, is no guarantee of survival. Embryos, in which the wound surfaces are com-

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