is a connection between wound healing and repair on the one hand and mast cells and histamine on the other.

In studies on pig, microscopy revealed a superficial necrosis to take place regularly in full thickness skin grafts. Pig skin differs from rat skin by containing only one main concentration of mast cells and histamine in the subepidermal layer of the dermis(13), whereas the mast cells and histamine in the rat are mainly located in an inner layer(2) close to the base of the graft. This closer relation to the vessels of the wound bed may be of importance to survival and the early phase of repair.

The present study does not allow the conclusion that histamine is important during the continued repair processes in skin grafts. However, the increase in histamine forming capacity demonstrated in wound tissue by Kahlson and co-workers(6) may suggest such a further influence. The secondary reduction of skin histamine in uninjured areas indicates that regeneration of the graft histamine may take place at the expense of normal skin histamine.

Summary. Analyses of histamine and water content performed on full thickness autografts of rat skin showed a considerable histamine release during the first 24 hours after operation. Simultaneously an increase in water content of the graft was measured. It is suggested that histamine may play a role in the early phase of repair and to survival of the graft, presumably by increasing vascular permeability.

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Antigen Stimulated Proliferation in Lymphoid and Myeloid Tissues from Immunized Rabbits.*† (29674)

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Earlier work from this laboratory has established that the uptake of radioactive thymidine by spleen cell suspensions from previously immunized rabbits is stimulated by addition of antigen *in vitro*(1). This response represents an *in vitro* counterpart of the cellular proliferation seen in the *in vivo* secondary immune response.

The purpose of the experiments reported here was to determine if similar antigen-de-

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pendent cell division could be demonstrated in cells from other lymphoid and myeloid tissues. The tissues studied were: spleen, mesenteric lymph nodes, popliteal lymph nodes, bone marrow, peripheral leukocytes, thymus, and alveolar macrophages. The response of the tissues to phytohemagglutinin-M was also investigated.

Methods. The procedures for immunization, preparation and incubation of cell suspensions, and measurement of thymidine incorporation were carried out as previously described(2).

Cell suspensions were prepared from rabbits which had been hyperimmunized to alum precipitated heterologous serum proteins, administered intravenously. Spleen, lymph nodes, and thymus were minced and sieved. Bone marrow cells were harvested from the humerus and femur. Peripheral leukocytes were harvested by the method of Skoog and Beck(3) and alveolar macrophages as described by Myrvik, Leake, and Fariss(4). The washed cell suspensions were incubated in a modified Eagle's medium and rate of DNA synthesis determined by measuring the uptake of titrated thymidine.

Results and discussion. Spleen, mesenteric and popliteal lymph nodes. The response of spleen cells has been described(1,2). The response of lymph nodes, whether obtained from the mesenteric or popliteal area, was in every way comparable. In the absence of the immunizing antigen there was a rapid decline in rate of DNA synthesis during the first 24 hours after which the rate remained relatively constant. In the presence of the immunizing antigen there was a stimulation of DNA synthesis which became pronounced in the second 24 hours (Fig. 1). Degree of stimulation measured in the second 24 hours was proportional to the logarithm of the antigen concentration (Fig. 2). The response was antigen specific and smaller responses were observed with cross-reacting antigens. As with spleen cells(5), antigen may be added as late as 48 hours and a strong response still obtained. Spleen and lymph node cells were strongly stimulated by phytohemagglutinin M (P.H.A.) (Fig. 3). Radioautographs of lymph node cells pre-labelled with a pulse of tritiated thymidine and incubated with antigen showed that the increased rate of DNA synthesis was associated with division of the responding cells. Approximately one per cent of the freshly isolated cells incorporated tritiated thymidine in a one hour incubation period. The cells that became labelled were principally large, undifferentiated cells. The rate of DNA synthesis in stimulated lymph node cell suspensions was sometimes greater and sometimes less than that of similar cultures of spleen cells from the same animal. The rate of DNA synthesis in unstimulated spleen cell suspensions was higher than that seen with lymph node, and this was presumably a consequence of the myeloid activity of the spleen.

TABLE I. Response of Bone Marrow Cell Suspensions to Homologous Antigen and PHA.

a.	Control		3749
	Antigen (μg/ml)	15	2804
		150	2504
		1500	1633
b.	Control		5567
	PHA	1.30 *	812
		1.300	3909
		1.3000	6505

* Dilutions of Difco Bactophytohemagglutinin M reconstituted in 5 ml.

Approximately 1×10^7 cells were incubated in 1.5 ml medium containing the additions indicated. Uptake of radioactive thymidine was measured in the period 24-48 hr. Figures represent counts per min per culture.

Bone marrow. The initial rate of DNA synthesis in bone marrow suspensions was very high compared with that of spleen or lymph nodes. No increase in DNA synthesis was seen when suspensions of bone marrow cells were incubated with the specific antigen. Instead, concentrations of antigen or P.H.A., which uniformly produced increases in DNA synthesis in spleen or lymph node cultures, actually depressed DNA synthesis in bone marrow cultures (Table I).

Peripheral leukocytes. Cultures of peripheral leukocytes were unaffected by the presence of the specific antigen. On several occasions P.H.A. was able to induce a modest increase in DNA synthesis. This, however, was not uniformly noted and the relative increase in DNA synthesis was small com-

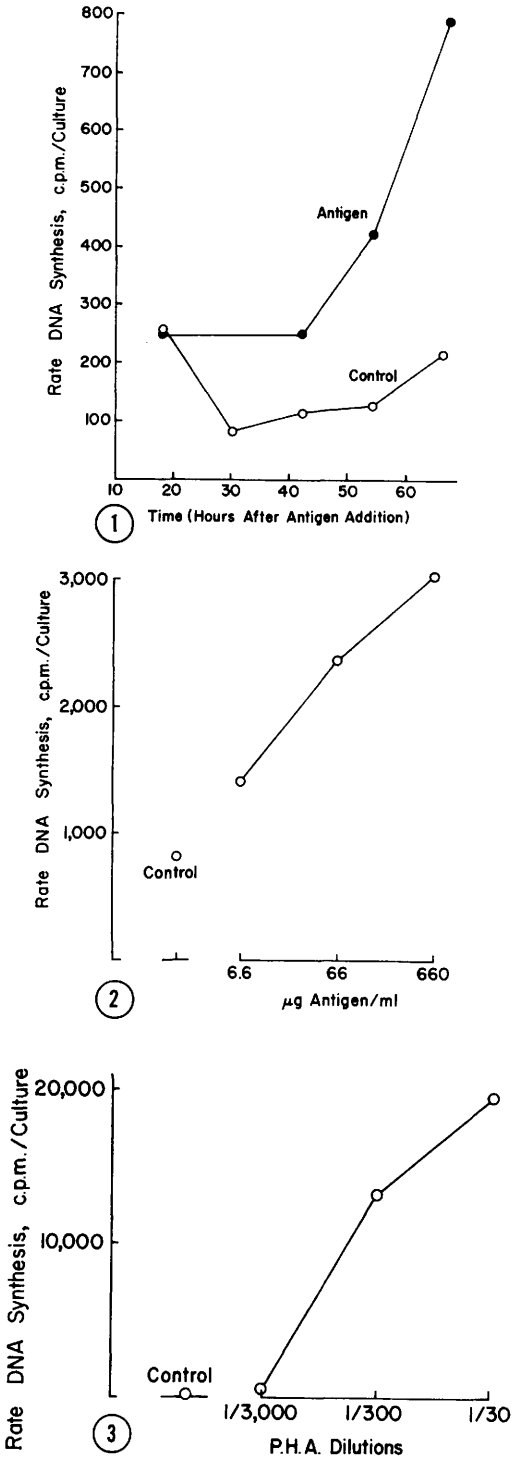

FIG. 1. Antigen stimulation of DNA synthesis in lymph node suspensions. Approximately 1×10^7 mesenteric lymph node cells were incubated in 1.5 ml of medium in presence or absence of antigen. Rate of DNA synthesis was determined by measuring uptake of radioactive thymidine in 10 hour incubation periods.

FIG. 2. Effect of antigen concentration on stimulation of lymph node cells. Approximately 1×10^7 mesenteric lymph node cells were incubated with concentration of antigen indicated. Uptake of radioactive thymidine was measured in the period 24-48 hours.

FIG. 3. Stimulation of lymph node cells by P.H.A. Approximately 1×10^7 mesenteric lymph node cells were incubated in 1.5 ml of medium containing the indicated dilutions of Difco Bactophytohemagglutinin-M, reconstituted in 5 ml. Uptake of radioactive thymidine was measured in the period 24-48 hours.

pared to that seen in the cultures of spleen or lymph node cells from the same animals. It should be stressed, however, that the low rates of synthesis of both control and stimulated cultures of peripheral leukocytes suggest that the culture conditions may have been inadequate for these cells.

Alveolar macrophages. Suspensions of alveolar macrophages did not incorporate significant levels of thymidine during incubation in the presence or absence of antigen, or when incubated with P.H.A.

Thymus. DNA synthesis in cultures of thymus cells was not influenced by the presence of the specific antigen to which the animal had been immunized. The rate of DNA synthesis at 24-48 hours was comparable to that seen in control cultures of spleen. Addition of P.H.A. to thymus cell cultures on occasion induced a small increase in DNA synthesis. High concentrations of P.H.A. inhibited DNA synthesis.

The responses observed were, in general, compatible with capabilities of tissues demonstrated by other techniques and in other experimental models (for review see 6). The failure of peripheral leukocyte suspensions to respond remains unexplained but may have been due to some inadequacy of the culture conditions. The inhibition of DNA synthesis by antigen (or P.H.A.) in bone marrow suspensions remains unexplained.

Summary. Suspensions of cells from spleen, mesenteric lymph nodes, popliteal lymph nodes, bone marrow, peripheral leukocytes, thymus, and alveolar macrophages were prepared from the tissues of hyperimmune rab-

bits. An *in vitro* secondary response, as measured by the uptake of tritiated thymidine, was elicited in spleen or lymph node cultures when incubated with the immunizing antigen. The uptake of tritiated thymidine represented an increased rate of DNA synthesis and was associated with division of the responding cells. A similar response was not seen in cultures of thymus cells, peripheral leukocytes or alveolar macrophages. The rate of DNA synthesis in cultures of bone marrow cells was depressed when they were incubated with either the immunizing antigen or phytohemagglutinin.

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Effect of Treatment with Gonadal Steroids on Conversion of ^{14}C-Estradiol to Estriol by Rat Liver.* (29675)

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Studies on urinary estrogens have indicated that the proportion of estriol in the total estrogen excretion differs between men and women(1) and between individuals of different race or environment(2). Attempts have been made to relate these differences to disease states, in particular, coronary disease(3,4).

Hagopian and Levy(5) have shown that estriol is formed from estrone by rat liver tissue *in vitro,* and the object of the present study was to compare the extent of the conversion of estrone to estriol by liver tissue from male, female, and castrated rats and to determine the effect on this conversion of treatment of the animals with sex hormones.

Materials and methods. Sprague-Dawley rats were housed in separate cages and fed Purina rat diet *ad libitum.* Castration was performed on the 14th day of age. Hormones in oil solution were injected into the rump twice weekly for 13 weeks beginning at the 22nd day of age. The total dosages per animal were: Testosterone propionate 65 mg; Estradiol-17β 6.5 mg; Progesterone 130 mg. The animals were killed by decapitation 108 days after the start of the hormone treatment. The livers were immediately excised and used for incubation.

In a typical experiment, liver slices weighing 2 to 5 g were incubated with ^{3}H-estrone or ^{14}C-estradiol-17β in Krebs-Ringer phosphate buffer at pH 7.4 in a Dubnoff shaker at 37°C under 95% O_2 and 5% CO_2 for 4 hours. Incubation was terminated by addition of acetone, and the incubate was placed in the refrigerator overnight. The filtered water-acetone extract was concentrated *in vacuo* to an aqueous residue, which was acidified with 10% HCl to pH 1 and extracted 6 times with 50 ml of chloroform. The chloroform extracted was evaporated to dryness and partitioned between water-methanol 3:7 and hexane. The hexane was discarded, and the aqueous methanol was evaporated to dryness. The residue was partitioned between chloroform and water. The chloroform extract was evaporated to dryness and dissolved in absolute methanol for chromatography.

In each extract the estrone, estradiol and estriol were separated on one of the following paper chromatographic systems:

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