positive donor cells in their spleens when sacrificed at 116 days after skin grafting. Lymphoid cells (30×10^6) from 3 comparable animals sacrificed at 130 days produced fatal homologous disease when injected into sublethally irradiated (A $\times$ T6)F_1 hybrids. The F_1 hybrids succumbed after a longer latent period (19-78 days) than after injection of lymphoid cells (30×10^6) from control strain A mice (10-26 days).

Seven strain A mice with short-term tolerance (15 to 55 days), did not immediately regain their immunological reactivity. A second test graft of the same origin placed after rejection of the first graft survived for periods remarkably similar to those for the first graft. When 2 comparable short-term tolerant mice were tested for chimerism, each of them contained a small percentage of donor dividing cells in the spleen at 33 days after skin graft rejection (7/103 and 1/64 respectively).

Four nontolerant mice (graft rejected within 14 days) did not display any detectable lymphoid chimerism (0/40 for each) when tested shortly after the rejection of the test graft.

In 2 strain A mice with complete tolerance to (A $\times$ T6)F_1 skin homografts for 26 months as a result of intravenous and intraperitoneal injection of F_1 spleen cells at birth, the graft remained fully preserved in spite of very low levels of lymphoid chimerism at the time of sacrifice.

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Observations on the Decomposition of Hemin by Fatty Acid Hydroperoxides.* (31476)

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The catalytic properties of hemoglobin and hemin in the oxidation of unsaturated fat, first observed by Robinson(1) in 1924, have since been the subject of extensive investigation(2). In aqueous emulsions of unsaturated fatty acids this catalytic action is exerted at the water-lipid interface in the presence of oxygen with subsequent decomposition of hemin(3,4). No hemin catalysis is observed in a homogeneous solution of linoleic acid in glacial acetic acid, dioxane or pyridine (4). It is not clear whether the destruction of hemin in a heterogenous medium is caused by free radicals formed in the process of peroxidation or by the action of preformed hydroperoxides. The absence of hemincatalyzed methyl linoleate oxidation in glacial acetic acid suggests that this medium is suitable for the study of hemin-hydroperoxide reactions

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TABLE I. Destruction of Rabbit Hemoglobin by Emulsified Esters of Fatty Acids and MLHP.

Ester used in reaction mixture*	O.D. at 410 mμ after 4 hr incubation
Methyl oleate	1.05
Methyl linoleate	.52
MLHP†	.32
None (Tween 80 only)	1.08

* Prepared by combining 2 ml of an aqueous emulsion containing 2 mg ester and 2 mg Tween 80 with 2 ml of an aqueous solution of hemoglobin (0.6 mg/ml).

† Methyl linoleate hydroperoxide (peroxide value 4780 meq/kg).

without interference from lipid free radicals. The results of such a study, reported here, show that in this medium hemin is rapidly decomposed in the presence of small concentrations of peroxides.

Experimental. Hemoglobin was prepared from rabbit erythrocytes according to the method of Drabkin(5) and hemin was prepared from hemoglobin by the acetone-hydrochloric acid procedure described by Anson and Mirsky(6). Hemin thus obtained showed the characteristic absorption maximum in the 410 mμ region (Soret band) and was stable when stored *in vacuo* at 4°C. In acetic acid solution the absorption maximum was observed to be shifted to 385 mμ. Methyl linoleate hydroperoxide (MLHP) concentrates were prepared from methyl esters of safflower oil fatty acids or methyl linoleate according to the procedure described by Kokatnur *et al* (7).

All the mixtures studied were incubated at 37°C in dim light for a period of 4 hours. The optical densities of the reaction mixtures were measured either at 385 mμ or 410 mμ in a Perkin-Elmer Model 202 recording spectrophotometer or a Beckman DU spectrophotometer. For purposes of comparison the destruction of hemoglobin by hydroperoxides in aqueous emulsion also was studied and analogous observations were made on a commercial sample of bovine hemoglobin.

Results. When incubated with an aqueous solution of rabbit hemoglobin at 37°C (pH 7.0) in an air atmosphere, emulsified methyl linoleate was observed to decolorize hemoglobin almost to the same degree as did methyl linoleate hydroperoxide (Table I). Neither methyl oleate nor the emulsifying agent (Tween-80) affected Soret band absorption during the 4 hours of incubation.

When a similar experiment was conducted using an acetic acid solution of fatty acid esters and hemoglobin no significant destruction of hemoglobin was obtained (Table II). However, MLHP decolorized hemoglobin to the same extent as observed in the heterogeneous medium used in the previous experiment.

To determine the optimum concentration of MLHP needed for complete destruction of hemoglobin in an acetic acid medium, various concentrations of MLHP were incubated with 4 mg bovine or rabbit hemoglobin in 4 ml acetic acid solution. The reaction mixture was placed in glass tubes which were flushed thoroughly with nitrogen, stoppered tightly and kept in a constant temperature water bath at 37°C for 4 hours. A linear relationship was observed between the concentration of MLHP and the amount of hemoglobin destroyed (Fig. 1). One mg of MLHP destroyed about 2 mg of hemoglobin from either source.

Since the disappearance of the characteristic red color of hemoglobin in the presence of MLHP is due to decomposition of the iron porphyrin moiety of the molecule, the concentration of MLHP necessary to destroy hemin is higher than that needed for decomposition of an equal weight of hemoglobin. Increasing amounts of MLHP were added to a known concentration of hemin in glacial acetic acid solution and the mixture was incubated at 37°C for 4 hours under nitrogen. The optical density of the samples then was

TABLE II. Destruction of Rabbit Hemoglobin by Esters of Fatty Acids and MLHP in Acetic Acid Solution.

Ester used in reaction mixture*	O.D. at 385 mμ after 4 hr incubation‡
Methyl oleate	1.02
Methyl linoleate	.92
MLHP†	.28
None	1.01

* Consisted of 10 mg methyl ester and 7.2 mg hemoglobin in 4 ml glacial acetic acid.

† Methyl linoleate hydroperoxide (peroxide value 4780 meq/kg).

‡ Read after 3 × dilution with acetic acid.

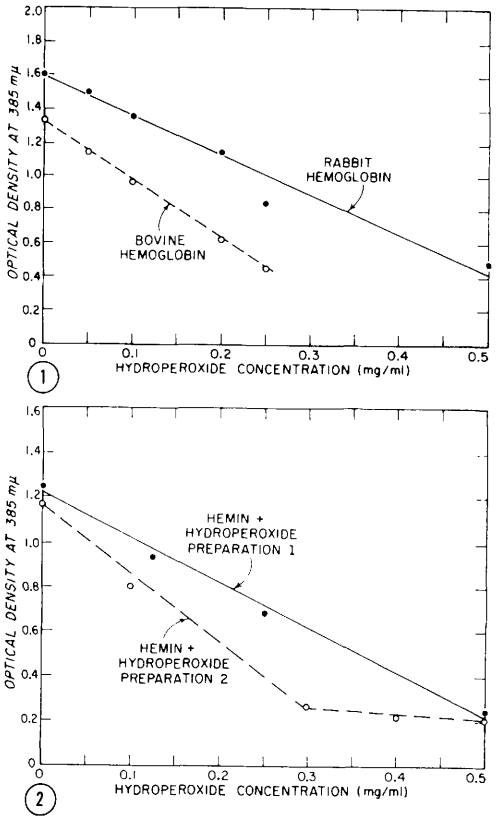

FIG. 1. Relationship between concentration of methyl linoleate hydroperoxide and destruction of bovine and rabbit hemoglobin in acetic acid solution. Reaction mixture contained 4 mg hemoglobin and 0-2 mg MLHP in 4 ml glacial acetic acid. Peroxide value MLHP was 4780 meq/kg.

FIG. 2. Relationship between concentration of methyl linoleate hydroperoxide and destruction of hemin in acetic acid solution. Reaction mixture contained 0.184 mg hemin and 0-2 mg MLHP in 4 ml glacial acetic acid. Peroxide values for MPHP preparations 1 and 2 were 4420 and 5920 meq/kg, respectively.

read at 385 mμ after 3-fold dilution with acetic acid. Calculation of the amount of pure MLHP required to produce half maximal change in optical density indicated that all the hemin was destroyed when the concentration of MLHP reached about 7 times the weight of hemin (Fig. 2). A similar result was obtained using 2 different hydroperoxide preparations. The same ratio was found to apply to the destruction of bovine heme as to rabbit heme. Fig. 2 also shows that the destruction of hemin is a linear function of hydroperoxide concentration.

Discussion. The destruction of hemin by hydrogen peroxide has been demonstrated and some of its decomposition products have been identified(8). The decomposition of hemin and hemoglobin in the presence of unsaturated lipids usually has been investigated under conditions in which the porphyrin compound has served as a catalyst for the oxidation of polyunsaturated fatty acids in an aqueous emulsion(3,9,10). Studies also have been conducted using mixtures of crude peroxides in an aqueous medium. Tappel(10) found that in a colloidal system 300 moles of hydroperoxides were needed to destroy one mole of hematin at 40°C. Dubouloz(11), using a different set of conditions, obtained a ratio of 50. In the homogenous system used in the present study, the destruction of 1 mole of hemin required only 4 moles of pure hydroperoxide.

The destruction of hematin catalysts which occurs during the oxidation of linoleate or during decomposition of linoleate peroxide has been attributed to random reactions between the hematin compounds and free radicals(10). However, these mechanisms do not satisfactorily account for the decomposition of hemin in acetic acid solution. As the peroxide preparations used in the present investigation were shown to be essentially free of linoleate, the destructive agents could not have been free radicals of this compound. Moreover, linoleate has been reported to be stable to hemin-catalyzed oxidation in an acetic acid medium (3), a finding confirmed in this study (Table II). It is unlikely that hydroperoxides would yield significant concentrations of free radicals in a strongly reducing solution of glacial acetic acid. MLHP was observed to be stable in this medium during the 4-hr incubation period employed in the present experiments, as indicated by the absence of any decrease in peroxide number or extinction at 235 mμ and by a lack of absorption at 280 mμ(10). The destruction of hemin under these conditions, therefore, is apparently due to the oxidative properties of hydroperoxides rather than to random encounter with free radicals formed in the process of their decomposition. This conclusion is also supported by the low concentrations of hydroperoxide required for

hemin decomposition.

The possible destruction of hematin compounds in the presence of oxidizing lipids *in vivo (e.g.,* the decomposition of cytochromes in the presence of peroxidizing mitochondrial lipids) is of interest from the standpoint of the lesions observed in animals deficient in vitamin E or exposed to ionizing radiation. The present results emphasize the strong oxidative properties of preformed peroxides toward such compounds. However, in the colloidal medium and under the pH conditions which prevail *in vivo* the role of fatty acid and peroxide free radicals may assume primary importance.

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Diuretic Effect of Angiotensin in the Chicken.* (31477)

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Pickering and Prinzmetal(1) demonstrated that renin was natriuretic in the rabbit, and Hughes-Jones, Pickering *et al*(2) suggested that this effect was due to a direct tubular effect of angiotensin. Recent studies by Laragh(3) and Langford and Pickering(4) have confirmed the diuretic effect of angiotensin in high doses in man and rabbit and again suggested that a tubular effect was present as glomerular filtration was reduced. Vander(7) in the dog found depression of distal sodium reabsorption when angiotensin was injected into the renal artery.

Nechay(5) used the Sperber chicken preparation to study theophylline diuresis. Substances introduced into the renal portal circulation *via* the leg vein will perfuse first and in the highest concentration the tubules of the ipsilateral kidney. An ipsilateral excess of electrolyte and water excretion was noted, suggesting that at least part of the effect of the xanthine compound was on the tubules. We have used the same approach to study the mechanism of angiotensin diuresis, and also we have studied the effect of angiotensin upon sodium excretion by conventional clearance techniques.

Method. White Rock hens weighing 2 to 3 kg were used. They were allowed to eat freely until the start of the experiment. An initial dose of 25 mg pentobarbital was used to supplement local anesthesia with 1% procaine. Funnels were sutured over each ureter, a catheter inserted into a leg vein, and the chicken suspended in a sling.

In the first group of experiments on 10 chickens, a 30-minute preliminary collection was made while 5% glucose/water was infused into a leg vein. Angiotensin II, Ciba, was then infused at 0.75 μg, 3.0 μg, and 6.0 μg/minute for the three 30-minute periods, the dose being increased stepwise in that order. A final 30-minute collection was made without angiotensin. Indigo carmine was infused into the leg vein at the start of most experiments. If it did not appear first and in highest concentration in the urine from the infused side, the chicken was discarded, for

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