sulted in delayed viral replication. They also found no virus in the lungs of untreated control infected mice on days 3, 4, and 5, but virus was present in high titer on days 2 and 6 after infection. Unfortunately only 3 animals were sampled at a given interval.

In our experiments no mice were completely free of virus in lungs at autopsy, although there was often less in the tissues of the treated group. Despite use of concentrated interferon and multiple pre-infection treatments, protection was not complete even with small doses of challenge virus. As it seems unlikely that this is due to mechanical differences in handling animals, we must assume that the mice varied in sensitivity to interferon and its production. Possibly, resistance developed only in those animals whose cells quickly responded to interferon and also had the capacity to produce endogenous interferon as a result of viral replication.

Interferon produced in chick embryo cells is also active in cells of a heterologous species, mouse lung tissue. Thus, although interferon from mouse lungs was less active than egg interferon in chick embryo tissue cultures infected with vaccinia virus, essentially equal potency was measured in the influenza-mouse lung model. The species specificity previously reported(9) apparently is reflected only as a quantitative difference.

The work of Isaacs and Hitchcock(10) suggested that augmentation of endogenous interferon, produced as a result of infection, with externally added interferon would reduce the mortality of influenza virus infection in mice.

Summary. Interferon prepared from influenza virus infected eggs or mouse lungs when intranasally administered protected mice against a mouse-adapted Type B influenza challenge. To be effective, the interferons had to be given before virus, and treatments delayed 24 hours after virus exposure served only to aggravate the infection. The concentration of virus used in the challenge was critical. The effect was best observed when the dosage was approximately 10 LD_{50}.

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Angiotensin II and Erythropoiesis.* (28707)

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The relationship of the juxtaglomerular (JG) apparatus to erythropoietin remains to be defined. Several investigators have suggested a relationship of the JG apparatus to erythropoietin production(1-3). Further, Fisher and Crook(4) have reported that angiotensin, indirectly a product of JG cells, has erythropoietic stimulating activity in

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hypophysectomized rats. Using polycythemic mice and hypophysectomized rats as assay animals, the erythropoietic stimulating activity of synthetic angiotensin II and angiotensin II made from a crude extract of hog kidney has been reexamined and compared to a concentrated sheep plasma erythropoietin. The results are presented here.

Methods and materials. Erythropoietic stimulating activity of the test substances was measured indirectly by incorporation of Fe^{59} in the erythrocytes of polycythemic mice and hypophysectomized rats.

Six-week-old CF-1 female mice were made polycythemic by the method of hypoxia described by DeGowin *et al*(5). The mice were given 1.0 mg of iron-dextran before being placed at one-half atmosphere for 3 weeks. The mice were then exposed to room air for 5 days before being injected with test substances. Injections of the test materials or saline were made intravenously on the fifth and sixth days posthypoxia and on the seventh day 0.5 μc of Fe^{59} citrate was injected. The red blood cell incorporation of radioactive iron was determined 48 hours later as previously described(6). The blood volume used for calculation of iron incorporation was estimated as 7% of the animal's body weight(5). Hematocrits were determined by the micro method and the results obtained from animals with a hematocrit of less than 52% were discarded.

Six-week-old hypophysectomized Sprague-Dawley rats† were used in other assays. The animals were fed a standard diet of laboratory chow supplemented with fresh vegetables, milk and bread. Two weeks following surgery, the animals were used for assay. The same injection schedule as outlined for polycythemic mice was followed. Total blood volume was assumed to be 5% of body weight. The results from rats with a hematocrit of less than 40% were not used.

The materials injected included: 1. Synthetic angiotensin II, 5 isoleucine,‡ designated hereafter as (A-S). The polycythemic mice received 100 μg/kg in each injection. The hypophysectomized rats received 50 μg/kg in each injection. 2. Angiotensin II produced from renin in crude extract of hog kidney,§ designated hereafter as (A-CE). The polycythemic mice received 35.5 μg/kg and the hypophysectomized rats 10 μg/kg in each injection. 3. Sheep plasma erythropoietin‖ 0.125 unit per injection, was given intraperitoneally to the polycythemic mice and 1 unit was injected intravenously in the hypophysectomized rats. 4. Finally, as a control, saline, 0.5 ml per injection was given both sets of assay animals.

TABLE I. Influence of Synthetic Angiotensin (A-S) and Erythropoietin on Erythrocyte Fe^{59} Incorporation of Polycythemic Mice.

Material inj	Route of admin	Fe^{59} incorporation in 48 hr (%) Exp 1 $Fe^{59} \pm$ S.D.	Exp 2 $Fe^{59} \pm$ S.D.
A-S	IV	.26 ±.20 (5)*	.18 ± .05 (5)*
Erythropoietin	IP	1.49 ±.40 (5)	3.10 ±1.70 (5)
Saline	IV	.22 ±.03 (5)	.12 ± .09 (4)

* No. of animals in exp.

In one study, normal human serum was incubated for 30 minutes with A-CE. The incubated material containing 7.5 μg of A-CE/kg was injected intravenously into polycythemic mice.

The pressor response of rats to the test substances was measured by the method of Boucher *et al*(7). The A-S, A-CE, and the incubated mixture of normal serum with A-CE all had marked pressor activity. The erythropoietin preparation showed no pressor activity in the rats used for assay.

Results. In the first series of experiments the erythropoietic stimulating activity of A-S was compared to saline and to erythropoietin. The results are shown in Table I. The A-S injections did not increase the red blood cell Fe^{59} incorporation in the polycythemic mice when compared to saline injected animals.

† Obtained from Hormone Assay Laboratory.

‡ Kindly supplied by Dr. William Wagner as Hypertensin-Ciba.

§ Kindly supplied by Dr. Oscar Helmer, Lilly Laboratories.

‖ Prepared by Armour & Co., Research Division and distributed through the Hematology Section, USPHS.

TABLE II. Influence of a Crude Extract of Angiotensin (A-CE) and Erythropoietin on Erythrocyte Fe^{59} Incorporation of Polycythemic Mice.

Material inj	Route of admin	Fe^{59} incorporation in 48 hr ± S.D. (%)
A-CE	IV	.37 ± .28 (5)*
Erythropoietin	IP	5.10 ±1.85 (5)
Saline	IV	.20 ± .06 (3)

* No. in parentheses refer to No. of assay animals.

Erythropoietin injections caused a significant increase in iron incorporation.

Similar results were obtained when A-CE was injected. The results are shown in Table II.

To determine a possible increase in erythropoeitic activity caused by incubation of A-CE with normal serum, the incubation mixture was injected in polycythemic mice. The results are shown in Table III. A-CE plus normal human serum resulted in a RBC iron incorporation of 0.25%. The Fe^{59} RBC incorporation resulting from A-CE and saline was 0.17%; from normal human serum 0.17%; and from 0.125 unit of erythropoietin 2.87%.

Previous studies from other laboratories have demonstrated an erythropoietic stimulating activity of angiotensin in hypophysectomized rats(4). These studies were repeated and the results are shown in Table IV. In hypophysectomized rats A-S, A-CE, and saline resulted in a RBC Fe^{59} incorporation of 11.8, 6.8, and 13.2% respectively. Erythropoietin increased the assay animals RBC iron incorporation to 40.3%.

Since it was possible that the A-S or A-CE acted as an inhibitor of erythropoiesis, additional experiments were performed in which the pressor materials were incubated with erythropoietin. The incubated materials were injected intravenously into polycythemic assay mice and the red blood cell radio-iron incorporation was measured. The saline injected control animals had a red blood cell iron incorporation of 0.31 ± 0.13% while animals injected with 0.5 unit of erythropoietin had an incorporation of 6.95 ± 1.62%. The incubation mixtures of 200 μg A-S/kg plus 0.5 unit erythropoietin and 71 μg A-CE/kg plus 0.5 unit erythropoietin resulted in red blood cell radio-iron incorporations of 7.72 ± 1.99% and 8.00 ± 2.44% respectively. Thus neither the synthetic angiotensin II or angiotensin made from a crude extract of hog kidney inhibited the erythropoietic response caused by a concentrated sheep plasma erythropoietin.

TABLE III. Influence of Injection of A-CE Mixed with Normal Serum and Erythropoietin on Erythrocyte Fe^{59} Incorporation of Polycythemic Mice.

Material inj	Route of admin	Fe^{59} incorporation in 48 hr ± S.D. (%)
A-CE + normal human serum	IV	.25 ±.07 (4)*
A-CE + saline	IV	.17 ±.07 (4)
Normal human serum	IV	.17 ±.02 (4)
Erythropoietin	IP	2.87 ±.91 (5)

* No. in parentheses refer to No. of assay animals.

TABLE IV. Influence of Synthetic Angiotensin (A-S), a Crude Extract of Angiotensin (A-CE), and Erythropoietin on Erythrocyte Fe^{59} Incorporation of Hypophysectomized Mice.

Material inj	Route of admin	Fe^{59} incorporation in 48 hr ± S.D. (%)
A-S	IV	11.8 ± 3.5 (6)*
A-CE	IV	6.8 ± 4.2 (6)
Erythropoietin	IV	40.32 ± 6.43 (3)
Saline	IV	13.2 ± 5.4 (6)

* No. in parentheses refer to No. of assay animals.

Discussion. The kidney plays a major role in production or activation of erythropoietin. The cellular localization of erythropoietin production remains to be defined. Several investigators have implicated the JG cells (1-3). Osnes has suggested that the kidney factor associated with erythropoiesis is connected with the granules of the JG cells and that these cells produce the factor or its precursor(1). Hirashima and Takaku studied the relationship between erythropoietin production and JG cells by estimating the JG granularity(2). After bleeding or treating rats with phenylhydrazine, the JG cells showed hypergranularity. After production of polycythemia, the cells showed hypogranularity. The authors concluded that the results gave indirect evidence for the secretion of erythropoietin by the juxtaglomerular cells of the kidney. Kaley and Demopoulous have

recently reported that treatment of rats with phenylhydrazine, cobalt or hypoxia sufficient to increase the erythropoietin stimulating factor, resulted in increases in the JG index. They concluded that the JG cells were responsible for the synthesis and release of erythropoietin(3). In humans, Reeves and his associates were able to directly correlate the hemoglobin values and JG cell counts in non-cirrhotics and in 12 cirrhotics with recent hemorrhage(8). However, Goldfarb and Tobian measured the JG index in rats rendered severely hypoxic for 12 hours(9) or hyperoxic for 21 days(10) and noted no change in JG granularity and probably no change in the rate of secretion of these cells.

What is known about the JG apparatus? Impressive evidence by extraction(11), micro-dissection(12), and fluorescein-tagged antibody experiments(13) indicate that renin comes from the JG apparatus. Renin reacts with plasma substrates to form angiotensin. The work of Fisher and Crook was of interest since they found that angiotensin increased red cell volume and radioactive iron incorporation in the red blood cells of hypophysectomized rats and also stimulated Fe^{59} incorporation in hypophysectomized-adrenalectomized rats(4). We were unable to duplicate these results. Using synthetic angiotensin, no increase in the red blood cell Fe^{59} incorporation, as compared to saline controls, was found in polycythemic mice, the most sensitive erythropoietin assay animals. Subsequently, hypophysectomized rats were used as assay animals and although these animals, too, were sensitive to purified erythropoietin they did not demonstrate an increase in Fe^{59} red blood cell incorporation following injection of A-S. Similar negative results were obtained using both A-CE and mixtures of A-CE with normal serum in an effort to determine if some activation product might be formed on incubation. Howevèr, the A-S and A-CE, when incubated with erythropoietin, did not inhibit the erythropoietic response of the assay animals.

The reason for the difference in these results and those reported by Fisher and Crook is not known, since the hypophysectomized animals were obtained from the same source and the angiotensin used in our experiments demonstrated marked pressor activity. It is of interest that Kaley and Demopoulous were unable to consistently increase erythropoietic stimulating factor production by administration of large doses of renin, as measured by the starved rat assay(3). It should also be noted that the sheep plasma erythropoietin was not pressor in the intact animal. This is further evidence that the products of the JG apparatus concerned with the renin angiotensin system are not related to erythropoietin. The negative results reported herein do not, of course, rule out the possibility that a second material is released by the JG cells.

Summary. 1. Polycythemic mice and hypophysectomized rats were used as assay animals to measure the erythropoietic stimulating activity of synthetic angiotensin II (A-S) and of angiotensin II made from a crude extract of the hog kidney (A-CE). 2. No increase in erythropoietic activity, as measured by the red blood cell incorporation of Fe^{59}, was found following injection of these substances. The assay animals were responsive to a concentrated sheep plasma erythropoietin. 3. These results suggest that the renin-angiotensin system does not encompass erythropoietin but do not rule out the possibility that a second material is released by the juxtaglomerular cells.

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The Non-Essentiality of Sodium Ions for Intestinal Calcium Transport.* (28708)

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In studies on the transfer of calcium across the intestinal wall, it has been established that an active transport system exists(1,2). Although the importance of the calcium transport mechanism in the overall calcium economy of the organism is unknown, it is generally accepted that a metabolically mediated system is available for control and modification of calcium absorption. Evidence further suggests that, at high concentrations of intestinal calcium, intestinal transfer is facilitated(3) but not necessarily by active transport as usually defined, *i.e.*, net movement against an electrochemical gradient by an energy dependent mechanism.

Sodium ions have been found essential for intestinal transport of glucose(4,5), amino acids(6) and other organic substances by both *in vitro* and *in vivo* techniques. Specifically in regard to glucose transport, Crane *et al*(4) suggested that sodium acts as an integral part of the glucose-carrier complex whereas Csaky(6) is of the opinion that sodium is necessary for the energy-dependent phase of the transport mechanism. The sodium ion is specifically required for the glucose system and cannot be replaced by lithium, potassium or choline(5,7).

Although calcium has been shown to be necessary for optimal functioning of some membrane transport systems(8-10), the essentiality of sodium ion for calcium transport has not been established. Schachter *et al*(11) observed that one-half of the Na^+ in the Wilson-Wiseman solution could be replaced by choline with no change in S/M ratios† for Ca^{45}; this was interpreted as showing that Na^+ does not compete with Ca^{++} for binding sites in the transport system.

The present studies were undertaken to investigate whether or not sodium is required for calcium transport by rat intestine *in vitro*; this should indicate whether there is a possible relationship between calcium transfer and transport of other substances by the intestine.

Methods. The *in vitro* procedures used herein were described previously(12) and were based upon the techniques of Schachter and Rosen(1) and Wilson and Wiseman(13). Duodenal segments were taken from young male rats of the Carworth strain, everted, washed in cold physiological saline, and blotted. The segment lengths were standardized at 8 cm. One end of the segment was tied with suture and 0.6 ml of incubating solution labeled with Ca^{45} was injected into the other end which was then tied, forming the closed sac. The sac was placed in a 25 ml Erlenmeyer flask containing 5 ml of the Ca^{45} labeled incubating solution, gassed for 1 minute with O_2 and incubated at 37°C for 1 or 2 hours. At the end of the incubation period, the solution within the sac was re-

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† Refer to *Methods* section for definition of S/M ratio.