TABLE I. Effect of Bile and Gastric Juice on Absorption and Liver Concentration of $Co^{60}B_{12}$ by the Intact Rat.

	No. of animals	Relationship of ligature to bile duct: Above	Below	No. of animals
Control	30	4.05 ± .33	7.50 ± .27	27
.5 ml gastric juice	20	6.65 ± .27	7.50 ± .36	20
.5 " bile	25	1.76 ± .42	1.74 ± .21	28
.5 " " + .5 ml gastric juice	20	4.98 ± .30	6.90 ± .60	25

Each figure is mean ± stand. error of mean in $\mu g \times 10^{-4}$ of B_{12}.

Dr. Paul S. Hagens using *Euglena gracilis*. His data showed that this lyophylized bile contained 95 $\mu\mu g$ of B_{12} per g of solids. This compares to a value of 4 $\mu\mu g/g$ in dog bile (5). Therefore, one ml of bile, 27 mg of solids, contains 8% as much B_{12} as was used in these experiments.

These experiments throw no light on why bile causes inhibition of B_{12} absorption in these animals; however, non-specific inhibitory binding of B_{12} would explain the results. It is known that bile will bind B_{12}(6). In these experiments, amount of intrinsic factor necessary to improve B_{12} absorption seems related to amount of bile present in the upper intestine. This effect would in part explain why isolation of potent pure intrinsic factor has been so difficult since minimal effective dose of intrinsic factor may be more related to the bile in the intestine than to amount of vitamin B_{12} ingested. It is possible that other intestinal secretions inhibit B_{12} absorption.

Summary. 1. In the intact rat the presence of bile in the small intestine inhibits B_{12} absorption. 2. The inhibitory effect of bile is prevented by simultaneous addition of rat gastric juice.

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Gamma Globulin and Immune Mechanisms in the Chick Embryo.* (26279)

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Green and Lorincz(1) stated that a serum protein with the properties of a natural antibody first appeared in the blood of chick embryos around the 17th day of embryonic development. This antibody, which is a part of the serum gamma globulin, seems to be responsible for destruction of the Krebs ascites tumor cells of mice injected intravenously into the chorio-allantois of chick embryos. Mouse tumor cells do not grow in hatched chicks. In connection with our findings(2) on the fate and behavior of mammalian skin heterografts transplanted to the chorio-allantois of the chick embryo, it was a natural sequence to learn whether gamma globulin placed directly on the embryonal membrane, or injected intravenously into the

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chorionic blood vessels, would have any possible influence on skin heterografts transplanted to the chorio-allantoic membrane. This investigation could also furnish additional information on the role of the serum gamma globulin in immune mechanisms.

Methods and technics. Skin grafts were taken from the flanks of rabbits and guinea pigs, and divided into pieces approximately 10 × 10 mm in size. The technic of preparation of the 10-day-old fertile chick eggs and application of the skin grafts to the membrane has been described(3).

Whole blood containing anti-rabbit gamma globulin was pooled from a group of hens previously immunized with rabbit spleen suspension. Serum protein fractions were obtained by the technic of Green and Lorincz(1) and final protein concentration of anti-rabbit gamma globulin was determined as 109 mg/ml. Anti-guinea pig gamma globulin was prepared in the same fashion. Concentration was 50.5 mg/ml. The guinea pig antibody fractions were found to agglutinate normal guinea pig red blood cells at 1:1,000 dilution.

The technic of Beveridge and Burnet(4) was used for injecting gamma globulin intravenously into the chorionic vessels of 85 chick embryos immediately prior to skin heterografting in a first series. In a second series of 104 embryos the gamma globulin was placed on the chorio-allantoic membrane adjacent to the skin heterografts. In a third control series 99 embryos received viable skin heterografts and no gamma globulin.

The mortality after grafting 288 eggs was 30%, but bacterial and mycotic infections caused an additional 15% loss despite the use of appropriate antibiotics. The eggs were sacrificed on the 4th, 5th, and 6th post operative days for histological sectioning. Daily stereomicroscopic examinations were made to determine viability of the grafts.

Results. The control eggs with skin heterografts and no gamma globulin exhibited the histologic and stereomicroscopic findings previously reported by us(2). Penetration of the chick blood vessels into the dense dermis of the guinea pig skin heterograft was not as deep or as extensive as in the rabbit skin heterograft.

After intravenous injection of 0.04 to 0.06 ml (2.0-3.0 mg) of concentrated guinea pig antibody serum no obvious effect on fate or behavior of the implanted guinea pig skin grafts could be observed either by stereomicroscopic or histologic observation. When dosage was increased to 0.15 ml (7.5 mg) no visible changes of the guinea pig grafts were observed.

The topical application of 0.05 ml of anti-rabbit gamma globulin (5.0 mg) to the ectodermal surface of the chick membrane failed to elicit any accelerated rejection of the rabbit skin grafts. There was no response to the rabbit skin graft after direct placement of 0.06 ml (3.0 mg) of guinea pig protein fractions to the chorio-allantois of the chick embryo.

Use of rabbit and guinea pig anti-serum by either direct application or intravenous injection appeared to evoke no significant cellular activity within the membranal stroma underlying the graft. Compared with the controls, there was no significant increase in infiltration of leucocytic, lymphoid-type, or large round cells with vesicular nuclei in the mesoderm as described by Billingham and Silvers (5).

Discussion. Administration of immune gamma globulin into the chick chorio-allantoic vessels or to the ectodermal surface of the membrane fails to produce detectable damage to skin heterografts.

Green and Lorincz(1) demonstrated that when a large number of tumor cells were injected intravenously into the chorionic vessels of the chick embryo none of the chicks succeeded in removing the cells. This suggests that the quantity of skin heterograft is too great to be effectively influenced by immune fractions. The areas where the foreign tissues are placed, whether within the embryo, or on the chorio-allantois, or in the yolk sac, may be responsible for the differences in immunological responses. Perhaps when the graft is situated on the chorio-allantoic membrane, the blood supply is not sufficiently great to furnish adequate antibody or complement for the cytolytic effect. Green and Lorincz(1) also postulated that the larger the quantity, the more resistant the tissue

was to a fixed quantity of antibody and that the degeneration depended on whether the tissue was growing within the body of the embryo or in the yolk sac. The nature of the tissues used (*i.e.*, skin, organs, cornea, etc.) and the species from which the tissues are obtained are also 2 important considerations. Billingham, Brent, and Medawar(6) demonstrated that the power to react against different antigens may mature at different times. According to these authors, "Birth itself is no guide to the time of origin of immunologic maturity."

Summary. The histologic findings reveal that rabbit and guinea pig anti-serum placed directly on the chick embryonal membrane or injected intravenously into the chorionic vessels fails to produce any detectable changes to skin heterografts transplanted to the chorio-allantoic membrane.

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Utilization of Injected and Orally Administered Amino Acids by the Rat.* (26280)

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In an effort to determine whether the gastrointestinal tract was an important site of the leucine-isoleucine antagonism in the rat (1,2,3), the effectiveness of parenterally administered isoleucine and valine in preventing the growth retardation due to a dietary excess of L-leucine was compared with that of isoleucine and valine included in the diet. Initial results indicated that the parenterally administered amino acids were ineffective, so the ability of parenterally administered isoleucine to prevent a simple isoleucine deficiency was determined. The growth response of rats fed an isoleucine-deficient diet and injected with isoleucine differed from the response of rats fed a lysine-deficient diet and injected with lysine. These observations and others on the effects of dietary and parenterally administered isoleucine and valine in overcoming growth retardation due to an excess of dietary L-leucine are reported.

Experimental. Male rats of the Holtzman strain were used throughout these experiments. Except for experiments on rats trained to eat a single meal daily, each group consisted of 6 rats weighing between 100 and 110 g and was fed *ad libitum*. Rats trained to accept a single daily feeding were fed a 15% casein-dextrin diet for only 2 hours each day for a period of 2 weeks(4). Their body weights at start of the experiments were between 210 and 230 g. Average initial weights for the groups within each experiment did not differ by more than one gram. All rats were supplied with water *ad libitum*, were housed in individual cages with raised screen bottoms and were weighed at a regular time daily.

All of the diets contained salt mixture, 5%

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