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)

PARI D. SPOLTER AND A. E. HARPER
Department of Biochemistry, University of Wisconsin, Madison

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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(5); corn oil, 5%; and vitamins in mg/100 g of diet as follows: thiamine, 0.5; riboflavin, 0.5; niacin, 2.5; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; Vit. B_{12}, 0.002; inositol, 10.0; Vit. C, 5.0; choline chloride, 150; Vit. A, 400 IU; Vit. D, 200 IU; and α-tocopherol, 10.0. The fat soluble vitamins were dissolved in the corn oil.

The lysine-deficient diet contained 30% of wheat gluten and dextrin as the carbohydrate to make up 100%; 0.4% of L-lysine · HCl was included in the control diet(6). The isoleucine-deficient diet contained 18% of blood meal and 0.25% of DL-isoleucine with dextrin to make up 100%; 1% of DL-isoleucine was included in the control diet(1). The amino acid diet contained all of the amino acids in the quantities that would be provided by 9% of casein except that values for L-isoleucine and L-valine were reduced by one-third. Values from Block and Weiss(7) were used for the amino acid composition of casein. Sucrose was used as the carbohydrate in this diet and was included to make up 100%; the supplemented amino acid diet contained adequate amounts of isoleucine and valine. The high leucine diet contained casein, 9%; DL-methionine, 0.3%; L-glutamic acid, 0.74%; and L-leucine, 5%; the supplemented diet contained L-isoleucine, 0.325%; and L-valine, 0.30%; instead of the glutamic acid(3).

All amino acid solutions used for injections were isotonic (0.306 osmolar) and the pH was adjusted to 7.5.

Food consumption of individual rats was recorded daily and volume of the amino acid solution to be injected was calculated to provide each rat with a slightly larger quantity of amino acids each day than was consumed by rats receiving the supplemented diet.

In the *ad libitum* feeding experiments animals were injected twice daily at 8:30 a.m. and at 5:30 p.m. Subcutaneous injections were made under the skin on the back of the necks of the animals and intravenous injections into the tail vein. Control animals were injected with an equal volume of physiologic saline solution. Trained rats were injected once daily at the end of the 2-hour feeding period.

Results. A comparison of effects of administering lysine in different ways to rats fed *ad libitum* a diet deficient in this amino acid is shown in Fig. 1. Rats receiving 0.4% of lysine in the diet grew an average of 50 g more in 10 days than rats fed the unsupplemented diet. When lysine was administered by injection twice daily to rats receiving the unsupplemented diet, rate of growth was approximately half that of rats receiving lysine in the diet. Growth rates of rats injected subcutaneously or intraperitoneally were the same; both grew an average of 24-26 g more than the group fed the unsupplemented diet. The difference between the growth of the group injected with lysine solution and that of the group receiving the unsupplemented diet and injected with saline solution was highly significant ($P<.001$).

A comparison of the effects of administering L-isoleucine and L-valine to rats fed the diet containing an amino acid mixture that was low in these 2 amino acids is shown in Fig. 2. These animals like those in the previous experiment were fed *ad libitum,* and were injected subcutaneously twice daily. Rats receiving the complete amino acid diet grew 28 g more in 12 days than those receiving the amino acid mixture that was low in isoleucine and valine. There was no noticeable effect of the subcutaneous injections of isoleucine and valine up to 6 days. From the 6th to the 12th day the group receiving the amino acid injections grew a little more rapidly but the difference at the 12th day was not statistically significant ($P>0.05$).

In a similar experiment with rats fed *ad libitum* an isoleucine-deficient diet containing 18% of blood meal and 0.25% of DL-isoleucine no significant difference was observed between the growth of rats injected twice daily with saline solution and that of a group injected with an isoleucine solution for a period of 19 days.

Fig. 3 summarizes results of an experiment in which rats trained to accept a single daily feeding (2 hr) were fed the isoleucine-deficient diet containing blood meal and were injected with isoleucine or saline solutions at

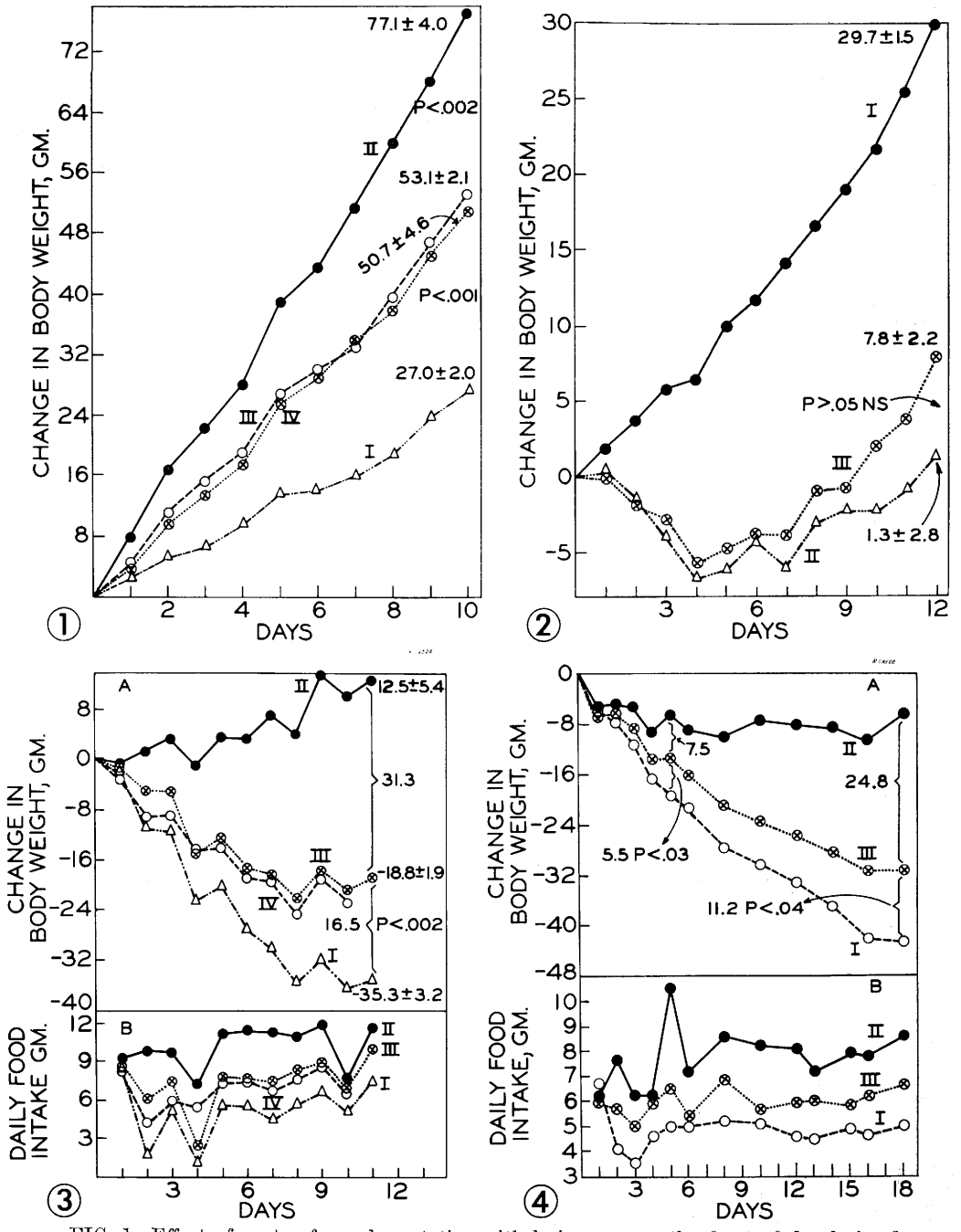

FIG. 1. Effect of route of supplementation with lysine on growth of rats fed a lysine-deficient diet *ad libitum*. I. 30% wheat gluten-dextrin diet, inj. with saline solution; II. as for (I) + 0.4% L-lysine • HCl in the diet, inj. with saline solution; III. as for (I) + 0.4% L-lysine • HCl inj. intraper.; IV. as for (I) + 0.4% L-lysine • HCl inj. subcut.

FIG. 2. Effect of route of supplementation with isoleucine and valine on growth of rats fed *ad libitum* a diet deficient in these 2 amino acids. I. Amino acid diet containing amino acids in quantities in 9% of casein; inj. with saline solution; II. as for (I) except that quantities of L-isoleucine and L-valine were reduced by one-third, inj. with saline solution; III. as for (II) + L-isoleucine and L-valine inj. subcut.

the end of the feeding period. The groups receiving isoleucine supplement by subcutaneous or intravenous injection grew significantly faster ($P<.002$) than those given injections of saline solution. However, the response of rats receiving isoleucine injections was only one-third that of rats receiving isoleucine in the diet.

In Fig. 4 are summarized the results of an experiment in which rats trained to eat a single meal daily were fed a diet high in leucine (9% of casein + 5% L-leucine). Isoleucine and valine were included in the diet of one group, and another received these amino acids by subcutaneous injection at the end of feeding period as in the previous experiment. Isoleucine and valine injections produced a significant growth response ($P<.04$) but again it was only one-third as great as that obtained when the amino acids were included in the diet. The response was significant ($P<0.03$) by the 5th day.

A further point of interest is that rats trained to eat a single meal daily (2 hours feeding every day) ate the same quantity of the isoleucine-deficient or the isoleucine-supplemented diet on the first day, but on the second day and thereafter the group receiving the isoleucine-deficient diet ate much less (Fig. 3). Rats fed the high-leucine diet or the high-leucine diet supplemented with isoleucine and valine (Fig. 4) also ate equal amounts on the first day and only on the second day did the food intake of the group receiving no extra isoleucine and valine fall. The appetite-depressing effects of isoleucine-deficiency and of excess leucine appear, therefore, to result from metabolic disturbances and not from low palatability of the diets.

Discussion. The observation that parenterally administered lysine is used more efficiently by the rat than parenterally administered isoleucine emphasizes that rates of catabolism of the individual amino acids in animal tissues differ considerably and that such differences are of much more significance when amino acids are administered parenterally.

Utilization of parenterally administered tryptophan(8), threonine(9) and lysine(10) has been reported but no systematic study of the relative rates of catabolism of individual amino acids administered by both routes appears to have been made.

Dalgliesh and Tabechan(11) observed that the overall rates of degradation of phenylalanine and tryptophan were similar, and about one-half that of tyrosine in the rat. Using chicks, Ousterhout(12) found the greatest rate of oxidation for labeled valine, this was followed by histidine, and threonine had the slowest rate of oxidation. Wu *et al.* (13,14) reported that concentration of N^{15} in ammonia and urea was higher after administration of labeled aspartate to human subjects than after administration of phenylalanine. Borsook *et al.*(15) found that mice injected with labeled amino acids oxidized 57% of the injected activity from leucine, 44% of that from histidine, 28% of that from glycine and 27% of that from lysine to expired CO_2 within 4 hours. Radioactivity in the non-protein fraction of the blood disappeared within one hour after glycine, leucine and histidine administration but a substantial amount of radioisotope was found in the 4-hour sample of blood from mice receiving lysine-C^{14}. Doty and Eaton(16) injected fasted dogs with glycine or lysine and observed that blood urea-nitrogen increased much less after lysine than after glycine administration. Bender(17) using rats obtained a much higher value for the net protein utilization of a lysine-free diet than for

FIG. 3. Effect of route of supplementation with isoleucine on growth of rats fed an isoleucine-deficient diet for only 2 hr daily. A: Growth; B: Food consumption. I. 18% blood meal + 0.25% DL-isoleucine in diet, inj. with saline solution; II. 18% blood mean + 1.0% DL-isoleucine in diet, inj. with saline solution; III. as for (I) + 0.75% DL-isoleucine inj. subcut. at end of feeding period; IV. as for (I) + 0.75% DL-isoleucine inj. intrav. at end of feeding period.

FIG. 4. Effectiveness of subcut. injections of L-isoleucine and L-valine in overcoming growth-retarding action of excess leucine in rats fed for only 2 hr daily. A: Growth; B: Food consumption. I. 9% casein + 5% L-leucine in diet, inj. with saline solution; II. as for (I) + 0.325% L-isoleucine + 0.30% L-valine in diet, inj. with saline solution; III. as for (I) + 0.325% of L-isoleucine + 0.30% of L-valine inj. subcut. at end of feeding period.

diets lacking any of the other indispensable amino acids. He also reported that animals survived for more than 6 months on a lysine-free diet.

Studies of the effects of delayed oral supplementation of diets with amino acids have led to the concept that for efficient synthesis of tissue proteins all of the essential amino acids must be supplied to the organism at close to the same time(10,18-23). The necessity for simultaneous availability of all of the essential amino acids indicates that amino acids are not stored to any appreciable extent in the body. However, in several experiments in which plasma amino acid concentrations of fasted dogs(24), fasted rats(25) and fasted chicks(26) were determined values for threonine and lysine were consistently higher than those for other indispensable amino acids. This may be further evidence of a slow rate of catabolism of these 2 amino acids and help to explain the relatively efficient utilization of injected lysine.

The present results indicate that differences in the extent of catabolism of individual amino acids may be greatly magnified if the amino acids are administered parenterally rather than fed as components of the diet. An awareness of this factor is important in studies of amino acid metabolism.

Summary. Rats fed a lysine-deficient diet *ad libitum* and injected subcutaneously twice daily with a lysine solution grew at about half the rate of rats receiving lysine in the diet. Rats fed *ad libitum* an isoleucine-deficient diet failed to respond when they were injected twice daily with an isoleucine solution. If rats were fed the isoleucine-deficient diet for a period of only 2 hours daily and were given subcutaneous injections of isoleucine at the end of feeding period their rate of growth was about one-third that of rats receiving isoleucine in the diet. Under the same conditions, injection of rats fed a diet high in leucine with isoleucine and valine resulted in a comparable improvement of growth. These observations indicate that the rate of catabolism of isoleucine is much faster than that of lysine in the rat and that the amount of an amino acid catabolized depends upon mode of administration.

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Interactions of Triethylenethiophosphoramide, Estrogen and Gonadotropin On the Ovary of Hypophysectomized Immature Rat.* (26281)

R. W. PAYNE AND THOMAS L. WHITSETT

Depts. of Pharmacology and Medicine, University of Oklahoma School of Medicine, Oklahoma City

The dynamic morphologic changes occurring in the ovary during the normal ovarian cycle and under experimental conditions have been reviewed by Hisaw(1). Interference with these processes by cytotoxic agents which act most effectively on rapidly proliferating cells offers interesting avenues of study. The present report is concerned with disturbances provoked by triethylenethiophosphoramide (Thio-TEPA) on the ovary of the hypophysectomized immature rat and the influences of a pituitary gonadotropic preparation and diethylstilbestrol on such effects.

Methods. The experimental animals were immature rats of the Holtzman strain, hypophysectomized at age of 24 days. Injections were begun immediately after hypophysectomy and repeated daily for a total of 4 doses. At autopsy, 48 hours after final injection, the ovaries were quickly weighed, preserved in 10% buffered formalin and subsequently prepared for microscopic study by the standard Hematoxylin and Eosin technic. Ovarian weight was corrected to a final body weight of 100 g.

Thio-TEPA in a saline solution was administered intraperitoneally. The partially purified sheep anterior pituitary gonadotropin (Gonadophysin®) was administered subcutaneously in doses of .2 mg dissolved in .2 ml of saline. Diethylstilbestrol in doses of 1 mg dissolved in .1 ml of sesame oil was injected subcutaneously.

Results. Thio-TEPA administered alone to the hypophysectomized animals produced a slight but quantitative reduction in ovarian weight. The intense stimulation of increased ovarian weight by relatively large doses of estrogen or pituitary gonadotropin was more precipitously antagonized by Thio-TEPA. Maximal ovarian stimulation with the combination of gonadotropin and estrogen provided the most striking example of quantitative inhibition of ovarian growth produced by increasing doses of the antineoplastic agent (Table I).

Thio-TEPA alone in the smaller doses produced destruction of granulosa cells and follicular atresia, predominantly in the larger follicles. However, as the dose was increased this effect became more evident in smaller follicles. Destruction of primordial follicles

TABLE I. Effects of Thio-TEPA on Ovarian Weight in Hypophysectomized Immature Rat.

Treatment*			No. of		Mean ovarian wt, mg/100 g body wt ± S.E.
Thio-TEPA	DES	Gonadophysin	Animals	Survivors	
			10	10	18.07 ± .45
.1			3	3	15.65
.15			16	8	14.20 ± .97
	1		14	14	63.95 ± 2.20
.05	1		3	3	56.20
.1	1		18	11	44.20 ± 1.33
.15	1		23	12	39.20 ± 3.42
.2	1		4	1	25.70
		.2	5	5	41.68 ± 5.92
.1		.2	11	11	38.60 ± 3.63
.15		.2	14	11	31.80 ± 4.16
	1	.2	18	18	167.20 ±14.87
.1	1	.2	15	13	91.21 ± 4.70
.15	1	.2	19	13	68.20 ± 5.35
.2	1	.2	4	1	40.60

* Daily dose in mg administered for a total of 4 doses beginning immediately after hypophysectomy.

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