mor cells and normal and malignant leukocytes, a maximal respiration at 0.5-3% CO_2 in the gas phase. Contrary to *Bicz*(4), *Seelich*(12) demonstrated a stimulatory effect upon normal but no effect upon leukemic leukocytes. *Seelich and Letnansky*(13) found endogenous respiration of Ehrlich ascites tumor cells 8% higher in Krebs-Ringer-bicarbonate solution than in Krebs-Ringer phosphate. *Kieler*(3) demonstrated in Yoshida cells a relation between CO_2 effect and metabolic condition of the cells. Cells with a high mitotic coefficient and low respiration showed a great increase in oxygen consumption in presence of CO_2, whereas cells with high respiration and low mitotic coefficient were less stimulated or even inhibited by CO_2.

It is not possible from our experiments to make definite conclusions as to the mechanism of inhibitory effect of CO_2 upon respiration. However, the experiments with butyrate seem to indicate a relationship to fatty acid metabolism. In this connection, *Miller* (14) found a stimulatory effect of bicarbonate upon incorporation of acetate-C^{14} into adipose tissue. *Gibson, Titchener and Wakil*(15) demonstrated in fatty acid synthesis an absolute requirement for bicarbonate, which seemed to play a catalytic role. It is now generally accepted that the initial step in fatty acid synthesis, as pointed out by *Brady*(16), is a fixation of active CO_2 to acetyl CoA with formation of malonyl CoA. In the equilibrium between fatty acid oxidation and fatty acid synthesis, nutritive and hormonal factors are known to play an important role. Whether this equilibrium is influenced by CO_2 remains to be shown.

Summary. The effect of CO_2 on respiratory metabolism of Ehrlich ascites tumor cells was studied with the Cartesian diver technic. In absence of substrate and in presence of glucose, pyruvate or oxalacetate, CO_2 had a pronounced inhibitory effect upon respiration. Only butyrate addition was found to neutralize CO_2 inhibition. The stimulatory effect upon respiration shown for pyruvate, oxalacetate and glucose (at a 1 mM concentration) in a CO_2 free atmosphere, could, when CO_2 was present in the gas phase, be demonstrated only for glucose.

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D-Amino Acids as Source of Non-Specific Nitrogen for Growth of Rats. (25870)

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Feeding of essential amino acids alone, each at its required level, will produce only limited growth of rats. Normal growth is obtained by supplementing such regimen with non-es-

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TABLE I. Composition of Amino Acid Mixtures.

Amino acid	Mixture-1*L* Form	g	Mixture-1*DL* Form	g	Mixture-2*L* Form	g	Mixture-2*DL* Form	g
Arginine	*L*. HCl	.93	*L*. HCl	.93	*L*. HCl	2.12	*L*. HCl	2.12
Histidine	*L*. HCl	.35	*DL*. HCl	.35	*L*. HCl	.79	*DL*. HCl	.79
Isoleucine	*L*	.46	*DL*	.46	*L*	1.04	*DL*	1.04
Leucine	*L*	.85	*DL*	.85	*L*	1.93	*DL*	1.93
Lysine	*L*. HCl	1.25	*DL*. HCl	1.25	*L*. HCl	2.84	*DL*. HCl	2.84
Methionine	*L*	.22	*DL*	.22	*L*	.50	*DL*	.50
Phenylalanine	*L*	.48	*DL*	.48	*L*	1.09	*DL*	1.09
Threonine	*L*	.51	*DL*	.51	*L*	1.16	*DL*	1.16
Tryptophan	*L*	.10	*DL*	.10	*L*	.23	*DL*	.23
Valine	*L*	.72	*DL*	.72	*L*	1.63	*DL*	1.63
Tyrosine	*L*	.38	*DL*	.38	*L*	.86	*DL*	.86
Cystine	*L*	.20	*L*	.20	*L*	.45	*L*	.45
$NaHCO_3$		.99		.99		2.51		2.51
Total		7.44		7.44		17.15		17.15

sential amino acids. Experiments in our laboratory(1,2) and elsewhere(3) have shown that the requirement for non-essential amino acids is relatively non-specific, since increase in level of essential amino acids or addition of a single or a few non-essential amino acids will also significantly stimulate growth of animals. Moreover, ammonium compounds and other simple nitrogenous substances, including urea, can be effectively used as nitrogen sources for biosynthesis of non-essential amino acids. Our object was to find out whether *D*-amino acids, as supplied in a racemic mixture of essential amino acids, can serve as source of nonspecific nitrogen. Besides its scientific interest, our study has some practical implications in view of the relatively low cost of *DL*-amino acids compared with expensive *L* enantiomorphs.

Methods. Weanling albino rats of Yale and Holtzman strains were used. Each animal was housed in separate cage and allowed to eat and drink *ad lib.* The rats were divided into experimental groups of 4 rats each and totaling same weight at onset of treatments. Body weight changes, food and water consumption of animals were recorded daily during 10-day experimental period. A basal nitrogen-free diet was compared with various amino acid diets prepared by inclusion of appropriate amino acid mixture and by adjusting percentage of dextrin. Composition of basal nitrogen-free diet has been described (4). Amino acid mixtures (Table I) were composed of essential amino acids, including cystine and tyrosine, and were patterned after the composition of the rat carcass(1). All amino acids were present in their natural *L* form in Mixture-1*L* and in the racemic form in Mixture-1*DL*, except for *L*-arginine and *L*-cystine. Mixture-2*L* and Mixture-2*DL* were comparable to above mixtures except that the level of each amino acid was approximately doubled. Mixtures-1*L* and -1*DL* were calculated to contain 0.93 g nitrogen while the latter mixtures contained 2.11 g nitrogen. Amino acids used were commercial products. A mixture of *L* with *D* alloisoleucine was purchased from Nutritional Biochemicals Corp., Cleveland, O. All other amino acids including racemic and *L* and *D* forms of isoleucine were obtained from Mann Research Lab., N.Y.

Results. Comparative growth response of rats fed natural and racemic amino acids is summarized in Table II. Feeding of a mixture containing essential *L*-amino acids each at its minimum level resulted in average weight gain of 12 g. When concentration of all essential amino acids was approximately doubled growth rate of rats was increased to 23 g. Feeding racemic mixtures of amino acids at either level, however, produced growth inhibition, which was accompanied by anorexia and by lowered food and nitrogen efficiency.

Further studies were conducted regarding the nature of growth inhibition. That animals lost slightly less weight on a diet containing a higher level of amino acids pointed

TABLE II. Comparative Growth Response of Rats to Natural and Racemic Amino Acids.

Series No.*	Nature of diet†	10 day wt gain (g)	Intake: Food (g)	Intake: Water (ml)	Intake: N. (g)	Efficiency: Food	Efficiency: N.	NNR‡
I	Nitrogen-free	−13 ± .2§	51	64		−.26		
	1 *L*	12 ± 1.2	75	127	.693	.16	17	36
	2 *L*	23 ± 4.0	71	152	1.498	.32	15	24
	1 *DL*	−11 ± 2.2	30	64	.277	−.38	−41	6
	2 *DL*	− 7 ± 2.5	31	80	.659	−.22	−11	9
II	Nitrogen-free	− 9 ± .7	35	40		−.27		
	2 *DL* (*L*-isoleucine allo free, *L*-leucine, *L*-valine)	19 ± 1.5	54	137	1.134	.35	17	25
III	Nitrogen-free	− 7 ± 1.0	34	61		−.22		
	2 *DL* (*L*-valine)	2 ± 2.0	33	75	.686	.05	3	13
	" (*L*-leucine)	− 1 ± .5	31	77	.654	−.02	−1	11
	" (*L*-isoleucine allo free)	21 ± 4.0	58	147	1.224	.35	17	23
IV	Nitrogen-free	−15 ± 3.2	40	56		−.36		
	2 *DL* (1.04 g *DL*-isoleucine)	0 ± 1.2	44	80	.928	.00		16
	" 2.08 g "	23 ± 3.0	62	162	1.376	.36	16	27
	" 4.16 g "	24 ± 1.2	63	171	1.468	.38	16	26
	" (.46 g *L*-isoleucine allo free)	25 ± .7	72	174	1.466	.34	17	27
	" 1.04 g *Idem*	30 ± 2.7	78	182	1.651	.38	18	27
	" (1.04 g *L* with *D*-allo-isoleucine)	28 ± 3.0	71	183	1.488	.39	19	29
	" (.52 g *L*-isoleucine allo free + .52 g *D*-isoleucine)	22 ± 2.0	68		1.424	.32	15	26
	" (isoleucine-free)	−18 ± 1.2	32		.635	−.55	−28	−5
	" (.96 g *DL*-leucine)	1 ± 1.5	45	90	.897	.02	1	17
	" (.96 g *L*-leucine)	−1 ± 2.2	46	86	.922	−.02	− 1	15
V	2 *DL* (.26 g *L*-isoleucine allo free)	1 ± .7	52	94	1.056	.02	1	
	" (*Idem* + .26 g *D*-isoleucine)	0 ± 3.3	49	91	1.005			
	" (.52 g *L*-isoleucine allo free)	21 ± 2.3		190				
	" (*Idem* + .52 g *D*-isoleucine)	20 ± 1.3		210				

* In series I, II, III, and V rats of Yale strain were used whereas in series IV Holtzman rats were employed.

† Modification of basal amino acid mixture is given in parentheses.

‡ $\frac{\text{Change in wt of test group + loss of wt of nitrogen-free group}}{\text{Nitrogen intake of test group}}$.

§ Stand. error of mean.

to the presence of a possible dietary deficiency rather than to a toxicity.

When leucine, isoleucine and valine in the racemic Mixture-2*DL* were replaced by their natural enantiomorphs, growth inhibition was overcome. Anorexia which accompanied growth inhibition was also reversed. Substitution of *L*-valine or *L*-leucine alone for their respective racemic forms did not affect growth of rats. Complete reversal of growth retardation was obtained, however, by substituting the *L* isomer of isoleucine for *DL*-isoleucine. Rats ingesting a diet containing this modified racemic mixture gained 21 g of weight which is comparable to weight gain of 23 g on a diet containing all amino acids in the *L* form.

Moreover food and nitrogen efficiency values included in Table II show that these mixtures are equally efficient.

Experiments were also designed concerning nature and possible causes of apparent isoleucine deficiency. Isoleucine has 4 possible optical isomeric forms. Those which were available to us were tested for their effect on growth of rats. These studies showed that growth inhibition could be overcome by replacing *DL*-isoleucine, fed at a level of 1.04 g/100 g diet, by 1.04 g as well as by 0.45 g of allo-free *L*-isoleucine. Feeding of 1.04 g *L* with *D* allo isoleucine produced growth comparable to ingestion of allo-free *L*-isoleucine fed at same level. It was further found that feeding of *D*-isoleucine together with the *L* isomer did not significantly alter growth of rats, and moreover, that growth inhibition on the racemic diet could be prevented by increasing amount of original *DL*-isoleucine.

It may be concluded that the observed isoleucine deficiency resulted from use of an isoleucine preparation which was actually a mixture of 4 isomers of which only L-isoleucine is biologically active(5).

In view of known antagonistic effect of leucine on utilization of other amino acids, especially isoleucine and valine(4,6,7), and that the rat can utilize appreciable amounts of *D*-leucine(4) there was still a possibility that the isoleucine deficiency in our study was aggravated by an excess of leucine in the diet. This was not, however, the case since no beneficial effect resulted when leucine level was decreased or when *L*-leucine was substituted for the racemic form.

Assuming that only 25% of the original *DL*-isoleucine *i.e.* 0.26 g/100 g diet was biologically active, and that presence of other isoleucine isomers does not affect utilization of *L*-isoleucine, one would anticipate that intake of 0.26 g *L*-isoleucine would be insufficient to support growth of rats. The experimental data (series No. 5) support the validity of this conclusion. It is also apparent from the results that *D*-isoleucine does not inhibit utilization of *L*-isoleucine.

Discussion. Since *D*-amino acids are not usually contained in foodstuffs, it has been frequently assumed that feeding of racemic mixtures of amino acids may be harmful. In favor of this view are occasional observations of growth inhibition in experimental animals fed such mixtures(8,9,3). The present study may furnish one possible explanation of how such a growth inhibition may occur.

The experiments confirm the previous finding of Greenstein *et al.*(5) that of 4 known isomers of isoleucine only the *L* enantiomorph, free of allo form, supports growth of rats. *DL*-isoleucine obtained commercially may come as a mixture of either 2 or 4 isomers and presence of the allo forms in such samples is often not stated. Use of such preparations in research may lead to erroneous conclusions.

All available evidence points against the idea that the observed isoleucine deficiency was aggravated by presence of unnatural isoleucine isomers in the diet or by a possibility of amino acid imbalance, which could have arisen from too much leucine in the diet.

The data show that rats are able to utilize *D*-amino acids as a source of nitrogen for biosynthesis of nonessential amino acids. In fact efficiency values indicate that in this respect they are equivalent to their natural *L* antipodes. Prior to this work, Phillips and Berg(10) observed that supplementation with *D* isomers of lysine, threonine, leucine, isoleucine and valine accelerates growth of rats beyond that observed on *L*-amino acids alone.

The observation that *D*-amino acids in the racemic mixture can furnish nitrogen for synthesis of non-essential amino acids is of fundamental importance, since it was customary in the past to disregard the nitrogen supplied by the *D* forms except those which could be easily inverted to the *L* antipodes. This will have a great effect on the ratio of essential to non-essential amino acid nitrogen in many older amino acid diets studied.

Summary. Rats fed a diet containing physiological amounts of essential *L*-amino acids but lacking non-essential amino acids grew at a slow rate. Growth rate was increased by increasing concentration of all essential amino acids. Feeding a mixture of *DL*-amino acids in comparable amounts resulted in growth retardation of animals. Growth in-

hibition produced by the racemic mixture was traced to an isoleucine deficiency resulting from inclusion of *DL*-isoleucine, which was actually a mixture of all 4 isomers, thus effectively diluting the active isomer. Substitution of *L*-isoleucine or a mixture of *L*- and *D* allo-isoleucine for the racemic compound in the *DL* mixture entirely overcame inhibition and produced growth comparable to that obtained with *L*-amino acids. The experiments provide evidence that *D*-amino acids can be effectively used as source of nitrogen for biosynthesis of non-essential amino acids.

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Cholesterol Estimation on Unmeasured Drops of Whole Blood.* (25871)

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Risk of future coronary heart disease in middle-aged men is related to concentration of cholesterol in serum measured in apparent health(1,2,3). Accordingly, blood cholesterol measurement is indicated, both in research programs to define relationships more precisely in populations, and in screening and possible preventive programs. Measurement of serum cholesterol is readily made though reports from routine laboratories indicate the need for control(4). But practical requirements limit wide application of cholesterol measurements to scattered populations and insurance applicants. Complications of storing and transporting liquid serum may be avoided by drying a measured amount (0.1 ml) of serum on filter paper, in which cholesterol may accurately be estimated at leisure in another laboratory(5). This requires drawing blood, separating and accurately measuring serum onto paper. These seemingly unexacting requirements are not readily met everywhere and, in fact, impede programs involving cooperation of general practitioners or untrained assistants. To simplify the procedure we studied what may be done with a few drops of finger tip blood, collected on filter paper. The result is a method, adequate for many screening and epidemiological purposes, that makes only trivial demand except at a distant analytical laboratory. Proceeding from the knowledge that drops of serum on filter paper, dried in room air for an hour or 2, may be successfully analyzed for cholesterol months later, trials were made with whole blood; this proved to be stable. The question, then, was to estimate amount of blood used when no measurement is made at time of drawing. Advantage may be taken of the relative constancy in whole blood, except in persons with frank anemia or polycythemia, of concentration of iron, sodium or simply the solids.

Method. Discs of filter paper 4.25 cm di-

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