variety of microsomal reactions. The long duration of the inhibitory action of ANIT requires further study, but it may be due to binding of the inhibitor to the endoplasmic reticulum membranes, a non-specific alteration of enzyme structure, or suppression of enzyme formation.

Although SKF 525-A inhibits a very wide variety of drug metabolizing reactions(12), the sulfoxidation of chlorpromazine is one of the enzymic pathways which seems unaffected (17). ANIT resembles SKF 525-A in its lack of inhibitory action on the sulfoxidation of chlorpromazine.

Summary. When alpha-naphthylisothiocyanate was administered orally to mice, the hepatic microsomal drug metabolizing activity for hexobarbital and aniline was significantly depressed 2 hours after treatment; the inhibition persisted for 9 days. Chlorpromazine metabolism was not affected. Phenylisothiocyanate and beta-naphthylisothiocyanate also inhibited some drug metabolizing pathways at 2 hours, but were neither as effective, nor as persistent, as alpha-naphthylisothiocyanate. Alpha-naphthylisothiocyanate and phenylisothiocyanate added directly to hepatic microsomes inhibited hexobarbital metabolism in concentrations as low as 10^{-5} M. Chlorpromazine metabolism was affected only when the concentrations were 10^{-3} M.

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Parathyroid Size and Uptake of Alpha-Aminoisobutyric Acid in Intact and Hypophysectomized Rats.* (30373)

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Parathyroid function appears to be regulated largely by changes in serum ionized calcium concentration(1), but the possibility that pituitary or other hormones may modulate this regulation requires further study(2). There are as yet no direct methods for measuring synthesis or secretion of parathyroid hormone *in vivo*. Previous studies(3-5) indicated that the parathyroid uptake of a non-metabolized amino acid, α-aminoisobutyric acid (AIB) could provide a useful index of parathyroid function both *in vivo* and *in vit-*

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ro. In the present study, parathyroid size and AIB uptake were measured in normal male and female rats at different ages and in young male rats after hypophysectomy with or without growth hormone treatment.

Methods. The methods employed have been described previously(3,5). Ten to 20 μc of AIB-1-C^{14} (3.5 mc/mM) were injected subcutaneously. Twenty-four hours later the animals were killed by decapitation and then AIB uptake by parathyroids, thyroids, pituitary and diaphragm was measured. Parathyroid wet and dry weights were determined on the Cahn Electrobalance after microdissection. Since the dry weight showed less variability and the mean dry weight/wet weight ratio was 0.25, the value for tissue water was taken as 3 times dry weight. For thyroid, diaphragm and pituitary, tissue water was taken as 0.75 times wet weight. As a measure of functional activity per unit mass, the ratio of AIB concentration in tissue water to that in serum was calculated as follows: T/S ratio $=$ T/(W $\times$ S), where T is the tissue content of AIB-1-C^{14} in disintegrations per minute (dpm), W is tissue water content in micrograms and S is dpm per microgram serum water. A parathyroid activity index (PAI), which takes into account both functional activity and gland size relative to body weight, was calculated as follows: T/(S $\times$ B) $=$ PAI where T is total content of AIB-1-C^{14} in disintegrations per minute (dpm) in both parathyroids, S is dpm per microgram of serum water and B is body weight in grams. The serum calcium, inorganic phosphorus(5) and total protein concentration (using an AO-Goldberg refractometer) were measured in each animal. Ultrafiltrable calcium(6) was measured in pools of stored serum from experimental animals and on fresh sera obtained from separate groups of male and female rats 4 and 8 weeks of age.

Intact rats. Groups of male and female Rochester Wistar rats were maintained from weaning on a standard chow diet until sacrifice at 4, 8, 19, and 44 weeks of age. Additional groups of male or female rats of the same strain were studied at 27, 45, 52, and 71 weeks. Experiments were performed in June and in December.

Hypophysectomized rats. Experiments were performed on stock Rochester Wistar rats 8 weeks old. The animals were shipped to the Hormone Assay Laboratories (Chicago, Ill.) for hypophysectomy or sham operation and returned in 2 to 3 days. *Experiment I (June)* —These animals were hypophysectomized or sham-operated at 6 weeks. One week later, daily injections of 0.25 mg purified bovine growth hormone (kindly provided by the Endocrine Study Section, Nat. Inst. Health) in 0.25 ml of isotonic NaCl solution were begun and continued for 8 days prior to sacrifice. The sham-operated and untreated hypophysectomized animals received NaCl alone. *Experiment II (December)*—Animals were hypophysectomized or sham-operated at 4 weeks and maintained without therapy until injections of growth hormone or saline were begun 12 days prior to sacrifice.

Results. Intact animals (Table I). Total serum calcium concentration showed no consistent change with age, sex or season despite a progressive increase in protein concentration with age and a higher value for total protein concentration in females than in males. The percentage of ultrafiltrable calcium for 9 pooled samples of stored serum from different experimental groups was 58% with a standard deviation of $\pm$ 5% while the value for 10 pooled samples of fresh serum from male and female rats at 4 and 8 weeks of age was 71% $\pm$ 8%. These values showed no consistent relationship with sex or differences in total protein concentration. Serum inorganic phosphorus concentration decreased progressively with age and was lower for females than for males of the same age.

The ratio of the dry weight of both parathyroids to body weight was constant in growing female rats (Fig. 1). In males, the parathyroid weight/body weight ratio fell between 4 and 8 weeks at a time of very rapid weight gain and subsequently remained constant and significantly lower than the value in females. Although the T/S ratio for AIB showed considerable variability there were no consistent differences ascribable to age, sex, or seasonal variation. The parathyroid activity index (PAI) was greater in adult females than in males, largely reflecting the higher

TABLE I. Parathyroid Function in Intact Male and Female Rats at Different Ages.

Group	Age, wk	Time of exp, June (J) or Dec. (D)	Sex	Body wt, g	Serum: Ca, mg/100 ml	Serum: PO_4, mg/100 ml	Serum: Total protein, g/100 ml	Parathyroids: Dry wt, μg*	Parathyroids: Wt/body wt, μg/g × 100	Parathyroids: T/S ratio	Parathyroids: PAI
A	4	J	♂	52 (50- 56)	9.4 ± .1	9.3 ± .6	4.6 ± .1	36 (30- 38)†	68 ± 3	7.9 ± .5	14.4 ± 1.5
B	4	D	♂	80 (66- 98)	9.6 ± .2	10.7 ± .2	5.5 ± .1	46 (28- 56)	57 ± 5	5.8 ± .6	10.3 ± 1.4
C	4	J	♀	50 (46- 52)	9.3 ± .1	9.2 ± .4	4.6 ± .1	31 (20- 46)	63 ± 8	10.1 ± .5	17.2 ± 2.7
D	4	D	♀	78 (72- 84)	9.4 ± .1	9.6 ± .2	5.3 ± .1	46 (32- 56)	59 ± 7	5.6 ± .5	9.9 ± .8
E	8	J	♂	228 (204-260)	9.5 ± .1	9.2 ± .6	5.9 ± .1	97 (94-100)†	42 ± 4	7.4 ± .6	7.6 ± .2
F	8	D	♂	239 (228-262)	9.2 ± .1	10.0 ± .5	5.5 ± .1	93 (70-116)	39 ± 4	6.6 ± .2	7.9 ± .8
G	8	J	♀	152 (142-164)	9.5 ± .1	7.1 ± .7	6.5 ± .1	96 (72-124)	64 ± 7	8.3 ± .3	14.1 ± 1.2
H	8	D	♀	155 (144-170)	9.6 ± .2	8.3 ± .2	6.2 ± .2	81 (64- 94)	52 ± 3	6.4 ± .6	10.2 ± 1.1
I	19	J	♂	334 (282-394)	8.9 ± .1	6.6 ± .6	6.4 ± .1	133 (120-166)	40 ± 1	6.8 ± .3	7.2 ± .5
J	19	J	♀	214 (200-224)	9.7 ± .2	5.7 ± .3	7.3 ± .2	126 (108-150)	59 ± 4	7.2 ± .3	11.4 ± .6
K	27	J	♂	374 (340-410)	9.2 ± .2	6.0 ± .2	6.8 ± .1	154 (122-178)	41 ± 2	8.2 ± .9	9.0 ± 1.0
L	44	D	♂	461 (415-514)	9.4 ± .2	6.5 ± .3	6.4 ± .1	203 (168-258)	44 ± 3	6.0 ± .6	8.4 ± 1.5
M	44	D	♀	262 (218-296)	9.4 ± .3	6.1 ± .5	7.4 ± .2	158 (114-212)	60 ± 6	6.7 ± .5	12.3 ± .1
N	45	J	♀	246 (226-260)	9.7 ± .2	4.8 ± .3	7.9 ± .2	169 (142-200)	61 ± 7	8.1 ± .4	13.5 ± 2.0
O	52	D	♂	418 (408-456)	9.0 ± .2	5.9 ± .4	6.9 ± .1	203 (162-246)	49 ± 4	6.2 ± .6	9.2 ± .8
P	71	D	♀	256 (206-282)	8.9 ± .1	5.4 ± .2	7.5 ± .2	160 (102-242)	63 ± 8	6.1 ± .2	12.1 ± 1.6

Group	Age, wk	Time of exp, June (J) or Dec. (D)	Sex	Body wt, g	Thyroid: Wet wt, mg	Thyroid: Wt/body wt, mg/g × 100	Thyroid: T/S ratio	Pituitary: Wet wt, mg	Pituitary: Wt/body wt, mg/g × 100	Pituitary: T/S ratio	Diaphragm muscle T/S ratio
A	4	J	♂	52 (50- 56)	4.8 (4.0- 5.8)	92 ± 4	2.5 ± .1	2.8 (2.4- 3.4)	53 ± 3	3.8 ± .2	9.5 ± .5
B	4	D	♂	80 (66- 98)	7.6 (6.1-10.4)	94 ± 3	1.8 ± .1	3.6 (3.0- 4.3)	44 ± 2	3.6 ± .2	6.8 ± .4
C	4	J	♀	50 (46- 52)	5.3 (4.2- 6.5)	106 ± 7	2.8 ± .1	3.2 (2.8- 3.4)	64 ± 3	3.6 ± .3	9.2 ± .2
D	4	D	♀	78 (72- 84)	8.0 (5.9- 9.0)	103 ± 6	1.8 ± .1	4.2 (3.4- 5.4)	54 ± 2	3.2 ± .3	7.3 ± .2
E	8	J	♂	228 (204-260)	15.3 (13.4-16.4)	68 ± 2	1.8 ± .1	9.3 (7.4-10.9)	41 ± 2	3.2 ± .1	6.6 ± .4
F	8	D	♂	239 (228-262)	16.5 (13.5-19.3)	69 ± 3	1.6 ± .1	9.3 (8.8-10.5)	39 ± 1	3.5 ± .2	6.0 ± .3
G	8	J	♀	152 (142-164)	12.9 (10.3-15.4)	84 ± 5	1.9 ± .2	10.8 (10.0-11.2)	71 ± 2	3.8 ± .3	6.9 ± .4
H	8	D	♀	155 (144-170)	11.4 (10.0-12.8)	74 ± 2	1.6 ± .1	10.6 (9.2-12.0)	69 ± 2	3.4 ± .2	5.4 ± .3
I	19	J	♂	334 (282-394)	23.6 (19.0-31.3)	70 ± 3	1.4 ± .1	10.5 (8.8-12.3)	31 ± 1	2.7 ± .1	5.7 ± .4
J	19	J	♀	214 (200-224)	15.0 (11.4-18.8)	70 ± 5	1.6 ± .1	16.2 (14.6-18.2)	75 ± 2	2.8 ± .1	6.0 ± .2
K	27	J	♂	374 (340-410)	19.3 (12.3-23.2)	51 ± 5	1.8 ± .2	10.5 (9.2-11.8)	28 ± 2	3.1 ± .2	6.6 ±1.0
L	44	D	♂	461 (415-514)	24.3 (20.1-30.6)	53 ± 3	1.3 ± .1	12.8 (12.0-13.8)	28 ± 1	2.6 ± .1	4.7 ± .2
M	44	D	♀	262 (218-296)	19.3 (16.8-26.4)	74 ± 5	1.3 ± .1	16.0 (13.0-17.7)	62 ± 4	2.9 ± .1	5.7 ± .5
N	45	J	♀	246 (226-260)	14.7 (10.8-18.4)	59 ± 4	1.6 ± .2	16.1 (14.8-17.8)	66 ± 4	2.6 ± .1	5.8 ± .2
O	52	D	♂	418 (408-456)	20.9 (19.2-25.8)	50 ± 3	1.2 ± .1	11.4 (10.3-12.6)	28 ± 2	2.8 ± .1	4.4 ± .1
P	71	D	♀	256 (206-282)	18.2 (13.2-23.2)	72 ± 6	1.3 ± .1	15.6 (10.2-22.5)	62 ±10	2.9 ± .1	5.1 ± .3

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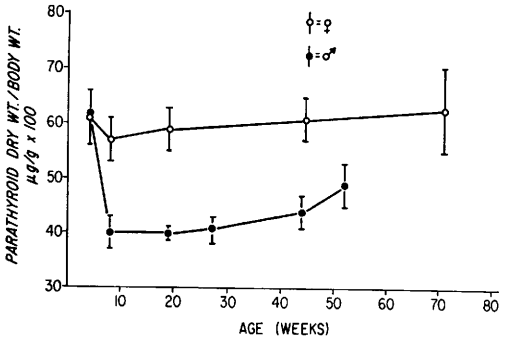

FIG. 1. Parathyroid size/body weight ratio in male and female rats of different ages. Data are mean and S.E. for groups of 5 to 11 rats. Values from experiments done in June and December were pooled.

parathyroid weight relative to body weight in females, but remained constant with age after 8 weeks in both sexes.

The thyroid gland showed some decrease in relative size and AIB uptake with age. The low tissue water/serum ratio for AIB uptake presumably was due to the fact that much of the tissue water is extracellular. The weight of the pituitary relative to body weight decreased with age in males. In females pituitary weight/body weight ratio was relatively constant with age and the values were significantly higher than in males. Pituitary T/S ratio for AIB was higher in 4- and 8-week-old animals than in older rats but was the same in males and females. The T/S ratio for AIB in diaphragm muscle declined with age and was the same in females and males.

Hypophysectomized rats (Table II). Hypophysectomy produced the expected arrest of growth and decrease in serum inorganic phosphorus concentration. Growth hormone reversed these changes at least in part. There were no significant differences in serum calcium concentration between hypophysectomized, sham-operated and growth-hormone treated animals. Variations in parathyroid dry weight occurred but these appeared to be related to body size, since the parathyroid weight/body weight ratio did not vary significantly. The T/S ratio for AIB was not altered by hypophysectomy or growth-hormone treatment. The PAI was actually higher for sham-operated rats than for the heavier, intact male rats studied at the same age. The expected decrease in T/S ratio for AIB of diaphragm muscle after hypophysectomy was observed in only one experiment. The thyroid gland was smaller in hypophysectomized rats but there was no consistent change in T/S ratio for AIB.

Discussion. The present study was undertaken with the expectation that parathyroid activity and size might decrease in older animals as the growth rate decreased. The absence of a direct relationship between parathyroid activity and growth rate suggests that the requirement for parathyroid hormone is not closely related to new bone formation or to bone turnover. The previous observation(7) that older rats show a lesser decrease in serum calcium concentration and in exchangeable calcium after parathyroidectomy than younger rats, may indicate that even though the older animal is secreting as much parathyroid hormone, changes in the metabolism and physical state of bone with age render it less responsive to that hormone. The present study did not include observations on very old animals. Such observations would be difficult to interpret in rats since in this species the epiphyses do not close and impaired renal function develops with aging(8) which may itself affect parathyroid activity.

The differences in parathyroid function observed between male and female rats are difficult to interpret. The relatively greater parathyroid activity in females cannot be ascribed to pregnancy or lactation since these animals were never mated. There was no difference in the amount of ultrafiltrable calcium although total protein concentration was higher in females; however, ionized calcium concentration was not determined. An effect of sex hormones on parathyroid function, whether

Values are mean ± 1 standard error for groups of 5 rats in the June experiment and 6 in the December experiment. Values in parentheses represent the range. See *Methods* for definition of T/S ratio and PAI.

* Dry weights of both parathyroids.

† Both parathyroids found in 4 of 5 animals in Group A and 2 in Group E. Values for parathyroid wt/body wt and PAI based on these animals only; T/S ratio calculated from entire group.

TABLE II. Parathyroid Function in Hypophysectomized, Sham-Operated, and Growth-Hormone Treated (0.25 mg/Day) Male Rats at Eight Weeks of Age.

Exp and treatment*	n	Body wt, g	Serum			Parathyroid				Thyroid			
			Ca, mg/100 ml	PO_4, mg/100 ml	Total protein, g/100 ml	Dry wt, μg	Wt/body wt, μg/g × 100	T/S ratio	PAI	Wt, mg	Wt/body wt, μg/g × 100	T/S ratio	Diaphragm T/S ratio
Exp I, June													
H	9	134 (118-154)	9.5 ± .2	9.1 ± .4	6.3 ± .1	60 (34- 84)	45 ± 4	7.1 ± .4	9.3 ± 1.0	10.1 (8.5-12.8)	75 ± 4	1.4 ± .1	5.6 ± .5
S	4	160 (128-197)	9.4 ± .2	12.2 ± .8	5.9 ± .1	89 (72- 98)	58 ± 8	7.0 ± .6	11.8 ± 1.7	15.2 (13.2-17.0)	96 ± 4	1.7 ± .2	6.2 ± .6
H + G	9	154 (116-180)	9.5 ± .2	10.2 ± .7	6.0 ± .1	80 (48-102)	53 ± 4	6.6 ± .6	10.4 ± 1.1	11.7 (8.8-16.2)	77 ± 6	1.7 ± .2	8.0 ± .7
Exp II, Dec.													
H	5	85 (74- 94)	9.1 ± .2	6.0 ± .7	6.5 ± .4	52 (34- 72)	61 ± 7	6.4 ± .9	11.4 ± 1.4	6.8 (5.4- 9.8)	80 ± 8	2.0 ± .3	7.8 ± 1.0
S	5	179 (128-236)	9.3 ± .2	10.2 ± .4	5.9 ± .1	94 (84-110)	56 ± 8	6.1 ± .3	10.0 ± 1.0	15.0 (11.8-18.6)	85 ± 6	1.7 ± .1	7.0 ± .6
H + G	5	120 (112-136)	9.2 ± .3	6.9 ± .3	5.7 ± .2	61 (34- 76)	51 ± 6	5.8 ± .9	8.7 ± 1.6	10.0 (8.2-11.0)	84 ± 5	1.7 ± .1	6.7 ± .7

Values are mean ± 1 standard error, for n animals in each group. Values in parentheses represent the range.
* H = hypophysectomy. S = sham operated. G = growth hormone.

direct or indirect, might explain the present data, and might bear on the clinical observation that bone demineralization is more common in postmenopausal women.

Seasonal variation in parathyroid function in man due to changes in ambient temperature have been suggested on the basis of changes in serum calcium and phosphorus concentration and in phosphorus clearance (9). Our data show no change in parathyroid activity in rats between June and December, but the environmental temperature probably varied less than in the studies in man.

Parathyroid function was found to be independent of any direct pituitary control in the present study. Differences in growth in hypophysectomized and growth-hormone treated animals were accompanied by proportional changes in parathyroid size. The data on hypophysectomized animals together with those on rats growing at different rates, confirm the direct relationship between serum inorganic phosphorus concentration and growth. These data provide further evidence that the parathyroids are not regulated by changes in serum inorganic phosphorus concentration.

Summary. Parathyroid function was assessed in intact male and female rats of various ages and in young, hypophysectomized male rats by measuring gland weight and uptake of the nonmetabolized amino acid, α-aminoisobutyric acid (AIB). Parathyroid weight/body weight ratio was higher in females 8 weeks and older than in males. The tissue water/serum concentration (T/S) ratio for AIB was unaffected by age, sex, or season. Hypophysectomy with or without growth-hormone treatment resulted in proportional changes in parathyroid weight and body weight and did not affect the parathyroid T/S ratio for AIB. Serum calcium concentration was constant with age while serum inorganic phosphorus concentration decreased and total protein concentration increased.

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Effects of Nicotinic Acid and Nicotinamide on Serum Cholesterol and Erythrocyte Nicotinamide Adenine Dinucleotide Levels of Rabbits.* (30374)

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The effectiveness of nicotinic acid (NA) and the ineffectiveness of nicotinamide (NAM) in lowering elevated blood cholesterol levels in humans are well established. Species differences in the activity of these agents do however exist and evidence will be presented indicating that in the cholesterol fed rabbit NAM is more effective than NA when given in equal dosage. Concerning the mechanism of action, Altschul(1) recently suggested that the cholesterol lowering effects of NA and NAM might be associated with their activity to increase erythrocyte nicotinamide adenine dinucleotide (NAD) levels. The results of an analysis regarding the effects of these agents on erythrocyte NAD levels in rabbits will be discussed in support of this hypothesis.

Materials and methods. Forty-eight male albino rabbits (2-3 kg) were used. The animals received 120 g daily of a commercial rabbit chow either as stock diet or supplemented with 1 g of cholesterol. NAM and NA were prepared as sterile solutions containing 200 mg/ml. NA was solubilized by neutralization with 2 N NaOH. Both agents were administered intraperitoneally in single daily dosage of 200 mg/kg. Blood for analysis of serum cholesterol and erythrocyte NAD levels was drawn from the central ear vein.

Serum cholesterol determinations were based on a modified Liebermann-Burchardt reaction using the stabilized Hycel† reagent. NAD was analyzed enzymatically with crystallized alcohol dehydrogenase‡(2,3).

Results. Response of serum cholesterol levels to treatment with NA and NAM. Twelve rabbits were placed on the cholesterol supplemented diet. Four of these animals received intraperitoneal injections of NA administered once daily during 5 consecutive days of each week, 4 received NAM in equal dosage and injection schedule, and the remaining 4 animals served as controls. After 4 weeks, injections were discontinued and the cholesterol diet changed to normal stock diet. Blood samples for determination of serum cholesterol were taken in weekly time intervals for a period of 6 weeks. Cholesterol values of the 3 groups were compared by means of analysis of variance(4). The observed changes and the statistical evaluation of the data are presented in Fig. 1 and Table I. While both NA and NAM were found to be effective in lowering serum cholesterol levels, NAM is apparently more potent in this respect.

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† Hycel, Inc., Houston, Texas.

‡ Boehringer Mannheim Corp., New York.