from 5 per min for *Noctiluca* to 1200 for a sponge choanocyte, though the lower frequency has been questioned by Lowndes (*10*). Gray (*4, 5*), using a mechanical stroboscope combined with a motion picture camera, later established a rate of 5–16 vibrations per sec in cilia from the gills of the mussel. An electric spark of a duration less than 0.0001 sec was used with a shutterless camera by Jennison and Bunker (*7*) to record movement of the cilia of clam gills. Hammond (*6*) measured the beat of cilia of several protozoa by means of a shuttered stroboscope and found, at 20°–24° C, a range from 6 or 8 per sec for *Vorticella* up to 42 per sec for *Stentor*. Lucas and Douglas (*11*) by direct observation with continuous light counted 2.2–5.2 beats per sec in cilia of a turtle's trachea. Lowndes (*8–10*) photographed moving flagella with a high speed motion picture camera which exposed 60 frames a sec with an exposure of 1/8000 sec, and recorded frequencies of about 7–12 beats per sec in several flagellates. Pease and Kitching (*12*), in a study of the effects of hydrostatic pressure on ciliary speed, used a variable speed, slotted, rotating disk similar to that used by most previous investigators. They reported that the cilia of mussel gills generally beat between 600 and 700 times per min. Except for Lowndes (*8*), who studied the sperms of an ostracod, apparently no investigator has measured the rate of flagellar vibration of sperms.

In the present study, human sperms were mounted in spermatic fluid at a temperature of 32° C and observed in dark field, illuminated by stroboscopic light.[1] The instrument used furnishes an intense light of very short duration, and can be simply and instantly regulated to produce flashes from 600 to 14,500 times per min. If the frequency of the flashes is the same as the frequency of the beat of the tail, one apparently motionless tail is visible. If the frequency of the flashes is twice that of the tail, there are apparently two tails. If the flash frequency is half that of the tail, then again one tail appears, but this rate will not be confused with the rate which obtains when flash and tail frequencies are the same if one bears in mind that, in the latter case, doubling of the flash frequency produces a double tail image.

Unlike most flagellated cells, the sperm cell does not move forward at a steady rate. Further, the tail does not beat with a simple harmonic motion. The sperm progresses in irregular jerks, each burst of speed lasting less than a second. It is during the moment of greater speed that the frequency of the vibrating tail can be determined, while between spurts of speed, the cell moves more slowly and the beat of the tail is so slow that I could not measure it stroboscopically. There is no perfectly rhythmic alternation of the periods of slow and fast beat, nor is there complete uniformity of behavior from cell to cell. Many cells cannot be "stopped" with stroboscopic light because they seem to be altering their speed so often that it is impossible to tune in on any frequency. The lowest flash frequency at which a single image could be observed was 14–16 per sec. With 25–28 flashes per sec, visibility improved because flickering was minimized, but during the moments when the tails were "stopped" they appeared double, due to the fact that the light was flashing twice during each period of vibration. The figures given are the extremes of a number of readings. That the double frequency is not exactly twice the single is probably due partly to inaccuracy of the method and partly to the fact that different cells were used for each reading, since I was not able to follow any single sperm long enough to make two readings on it.

It is interesting to find that the tails of human sperm cells have a frequency of beat which is rather close to that of the cilia or flagella of clam gills or monads; but such a result is to be expected, since, in spite of the fact that these various cells are far separated in organic history, they are still of so much the same order of magnitude that the surprise would have come if they had proved to move at radically different speeds.

[1] General Radio Company, Cambridge, Massachusetts.

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Action of Carboxypeptidase Toward Peptides Containing Unnatural Aromatic Amino Acids[1]

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Since β-2-thienylalanine has been found to inhibit the growth of certain organisms through interference with the metabolism of its analogue, phenylalanine (*2, 3*), it seemed of interest to prepare peptides containing this and other unnatural aromatic amino acids, and to determine whether these peptide analogues would be antagonistic to the action of isolated proteolytic enzyme systems, as well as to the growth of microorganisms.

Carboxypeptidase from beef pancreas was selected as the enzyme to use in this study because it displays maximum activity toward substrates derived from aromatic amino acids, and because it can be isolated readily in pure form. Carbobenzoxyglycylphenylalanine (I) is hydrolyzed by carboxypeptidase more readily than any other synthetic peptide, and its racemate has been suggested as

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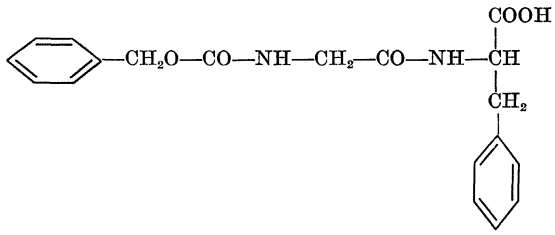
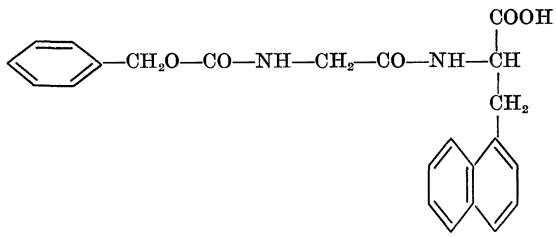
a standard for measurement of carboxypeptidase activity (*5*). Therefore, the compounds herein reported were fashioned after this peptide, and differ from it only by replacement of the benzene ring with other aromatic rings.

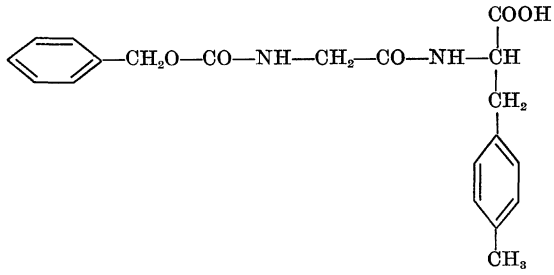
The following compounds have been synthesized in racemic form: carbobenzoxyglycyl-β-2-thienylalanine (II), carbobenzoxyglycyl-β-1-naphthylalanine (III), carbobenzoxyglycyl-β-2-naphthylalanine (IV), and carbobenzoxyglycyl-*p*-methylphenylalanine (V). At this time we wish to report the action of carboxypeptidase toward each of these compounds; the syntheses will be reported elsewhere.



Carbobenzoxyglycylphenylalanine (I)



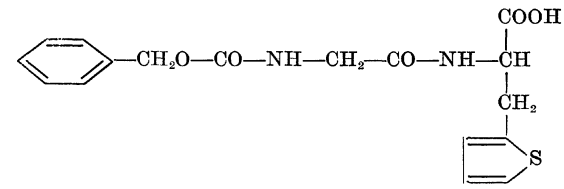
Carbobenzoxyglycyl-β-1-naphthylalanine (III)



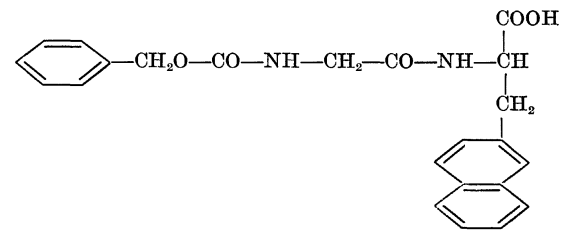
Carbobenzoxyglycyl-*para*-methylphenylalanine (V)

Table 1 lists the results of the hydrolytic studies. From these data it is possible to conclude the following: compounds (III) and (IV), containing the large naphthalene ring system, are quite resistant to hydrolysis; compound (II), containing the thiophene ring, is hydrolyzed at about one-half the rate of its benzene analogue, according to the initial velocity constants; compound (V), containing the *p*-methylphenyl group, is hydrolyzed about as readily by carboxypeptidase as is the typical substrate (I) in which the phenyl group is unsubstituted.

Stahmann *et al.* (*6*) found that certain peptides which resisted hydrolysis would inhibit the hydrolysis of a typical substrate when present in equimolar amounts. The data presented in Table 2 show the inhibitory effect of carbobenzoxyglycyl-β-2-naphthylalanine (IV) added in equimolar amounts to carbobenzoxyglycylphenylalanine (I). Simultaneous hydrolysis was carried out on (I) and



Carbobenzoxyglycyl-β-2-thienylalanine (II)



Carbobenzoxyglycyl-β-2-naphthylalanine (IV)

(IV) separately, under the same conditions, for comparison.

The carboxypeptidase used in this work was prepared by the method described by Anson (*1*). The enzyme concentrations given are based upon the rate of hydrolysis of racemic carbobenzoxyglycylphenylalanine (*5*). Each hydrolytic study was carried out in the following typical manner: 178 mg racemic carbobenzoxyglycylphenylalanine was suspended in 2 ml phosphate buffer, pH 7.6, and solution was effected by making distinctly pink to phenolphthalein with 1 *N* NaOH. The pH was reduced by the addition of 0.5 *N* acetic acid until the pink color just disappeared. The proper amount of enzyme suspension was added and the volume diluted to 5 ml. A 1-ml sample for the blank was immediately withdrawn and added to 9 ml of absolute alcohol, then reserved for subsequent titration. Hydrolysis was carried out at 37° C. The rate of hydrolysis was determined by titration of 1-ml samples by the alcohol titration method of Grassman and Heyde (*4*), using *N*/100 NaOH, with phenolphthalein as the indicator. The substrate concentrations were in each case 0.10 mM of the racemic compound per ml; the percent hydrolysis was calculated on the basis of the concentration of the L-isomer.

TABLE 1

RATE OF HYDROLYSIS OF SYNTHETIC PEPTIDES BY CARBOXYPEPTIDASE

Substrate	Enzyme concn. 10^{-4} mg N/ml	Time min	Hydrolysis %	Velocity constant, K* 10^{-3} min^{-1}
Carbobenzoxyglycylphenylalanine (I)	1.5	70	27	1.9
		140	50	2.1
	2.7	30	17	2.6
		60	46	4.4
		130	63	3.3
Carbobenzoxyglycyl-β-2-thienylalanine (II)	1.5	70	13	0.9
		140	19	0.6
		255	31	0.6
Carbobenzoxyglycyl-β-1-naphthylalanine (III)	2.7	30	0	
		60	2	
		130	2	
	7.0	25	0	
		135	2	
Carbobenzoxyglycyl-β-2-naphthylalanine (IV)	3.4	30	1	
		90	6	
		8 hr	11	
	13.6	23	2	
		70	9	
Carbobenzoxyglycyl-p-methylphenylalanine (V)	2.7	30	17	2.6
		60	25	2.0
		130	52	2.4

* $K = \frac{1}{\text{min}} \log_{10} \frac{100}{100 - \%\ \text{hydrolysis}}$.

TABLE 2

INHIBITION OF HYDROLYSIS OF CARBOBENZOXYGLYCYL-PHENYLALANINE BY CARBOBENZOXYGLYCYL-β-2-NAPHTHYLALANINE

Substrate	Enzyme concn. 10^{-4} mg N/ml	Time min	Hydrolysis %
Carbobenzoxyglycylphenylalanine	13.6	23	63
		70	73
		17 hr	79
Carbobenzoxyglycylphenylalanine plus carbobenzoxyglycyl-β-2-naphthylalanine	13.6	23	15
		70	54
		17 hr	75
Carbobenzoxyglycyl-β-2-naphthylalanine	13.6	23	2
		70	9

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Uptake of Radioactive Iodine by the Thyroids of Underfed Rats[1, 2]

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Thyroid activity in rats is depressed during starvation, an effect which has been attributed to a decrease in thyrotrophic function by the anterior pituitary (*3*, *4*). From a quantitative aspect, a recent report from this laboratory on the effects of thiouracil on the thyroids of starved rats and mice indicated that the decrease in thyroid activity may be directly proportional to the reduced body weight of these animals (*1*). In other words, thyroid activity appeared to remain unchanged in starved rats when computed on a body weight basis.

We decided to test this finding further by administering radioactive iodine to starved rats and comparing their thyroid uptake with that of controls fed ad libitum.

TABLE 1

EFFECTS OF UNDERFEEDING ON SURVIVAL, GROWTH, AND THYROID WEIGHTS OF RATS

Group	Orig. No. per group	Final No. per group	Avg orig. body wt in g	Avg final body wt in g	Avg thyroid wt in mg	Avg thyroid wt/100-g body wt in mg
Controls, fed ad lib.	10	10	147.0	169.5	13.79	8.13 ±* 0.29
Fed ¾ ad lib. .	10	10	146.6	148.5	10.34	7.00 ± 0.42
Fed ½ ad lib. .	10	10	145.5	126.6	8.97	7.07 ± 0.55
Fed ¼ ad lib. .	10	8	145.4	111.0	8.60	7.71 ± 0.23
No feed	10	4	146.0	87.0	7.20	8.24 ± 0.52

* Standard error of mean.

Fifty young female rats of the Sherman strain, weighing approximately 145 g each, were divided into five groups of ten each and were started on ad libitum, ¾, ½, ¼, and no-feed regimens. The ¾, ½, and ¼ feed-allowance levels were computed from the daily ad libitum feed consumption of the control group. The ration consisted of ground Purina Laboratory Chow. All rats were maintained in an air-conditioned room at a temperature of 75° F.

The unfed group was sacrificed at the end of 7 days, and the other four groups at the end of 14 days. Eight hours prior to sacrifice, each rat was injected intraperitoneally with 0.2 ml of carrier-free I^{131} (radioactive) estimated to contain approximately 2 μc. The thyroid of each sacrificed rat was removed, immediately weighed on a Roller-Smith balance and placed on the center of a

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