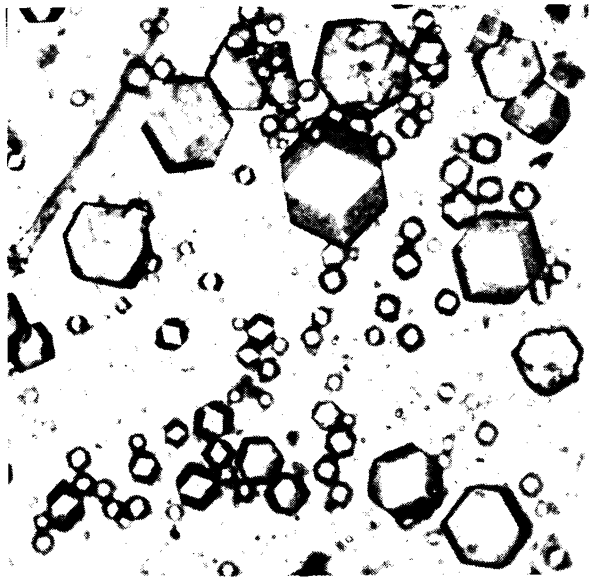

FIG. 1. Crystalline HGF.

mercial purification of insulin served as starting material. An initial precipitation at pH 6.7 yielded a material of considerable hyperglycemic activity and relatively free of insulin. Additional purification was achieved by collecting the fraction that separated between 50 and 76% acetone. Successive fractional precipitations at controlled pH in acetate and phosphate solutions resulted in a highly purified preparation that was relatively soluble in water but largely insoluble in presence of electrolytes. Administration of 0.15 μg/kg body weight of this material into cats gave a 30 mg% increase in blood sugar. A solubility curve indicated that the preparation was about 70% pure. Carboxyl-terminal amino acid analyses performed on the purified sample according to the method of Akabori (*5*) yielded essentially a single amino acid.

The highly purified preparation was dissolved in a buffer solution at alkaline pH. The precipitate obtained after centrifugation at 64,000 g was removed, and the supernatant solution was allowed to stand in the refrigerator overnight. Figure 1 shows a photomicrograph of crystals formed under these conditions.

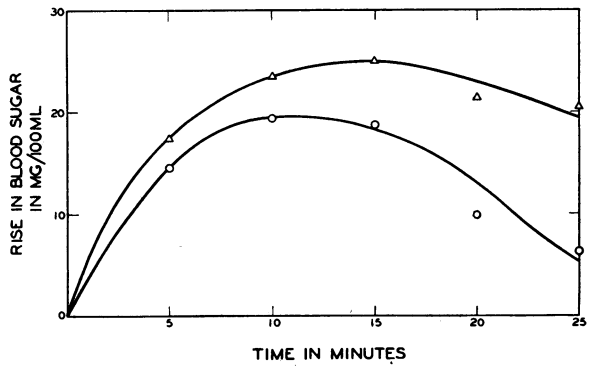

FIG. 2. Effect of HGF on blood sugar: triangles, average response of 6 cats after intravenous injection of 0.1 μg of HGF/kg body weight; circles, average response of rabbits after intravenous injection of 1.5 μg of HGF/kg body weight.

The crystalline material is relatively insoluble in cold water and gives positive biuret, Folin-Ciocalteu (phenol reagent), and Sakaguchi tests. The ultraviolet absorption curve shows a maximum at 278 mμ and a minimum at 250 mμ. The crystals belong to the isometric system and appear as rhombic dodecahedra. They contain only traces of zinc. Biological activity of the crystalline material was determined in both cats and rabbits. Results of these tests are presented graphically in Fig. 2.

Chemical and physical chemical properties, amino acid composition and analyses, and other characteristics are currently being determined with the crystalline material. This work along with detailed procedures for isolation will be published elsewhere.

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Failure of Cyanide to Inhibit β-Amylase

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According to Roy and Underkofler (*1*), treatment of malt extracts with 0.1% sodium cyanide at 30° for 1 hr had no effect on the α-amylase activities but lowered the saccharogenic activities of these extracts; NaCN completely inhibited the β-amylase activities of extracts from wheat, barley, and soybean, and of solutions of commercial β-amylase. The apparent survival of β-amylase activities in the malt extracts, according to them, was due to the limited dextrinase activities which resisted the cyanide treatment. In their experiments no attention was paid to the pH of the cyanide-treated enzyme solutions. Sodium cyanide is a strong base, and it can easily be supposed that the pH values of the NaCN-treated enzyme solutions would shift far to the alkaline side of the reaction. Furthermore, it is well known that in nonspecific inhibition the degree of inhibition is dependent on the relative concentrations of inhibitor and active centers in the enzyme molecule. From the foregoing considerations, it seemed of interest to reëxamine the effect of cyanide on the α- and β-amylase activities of various origins.

Potassium cyanide, in place of sodium cyanide, was used in this experiment. The control test was run simultaneously without added inhibitor, and tests were made with 0.1% HCN (neutralized to pH 5.0) and with 0.1% potassium carbonate, respectively. Potas-

TABLE 1

EFFECT UPON AMYLOLYTIC ACTIVITY OF PRETREATING AQUEOUS EXTRACTS OF MALT AND TAKA-DIASTASE WITH 0.1% HCN, KCN, OR K_2CO_3*

Sample	Protein content	α-Amylase activity				Total saccharogenic activity			
		Control	Treated with 0.1%			Control	Treated with 0.1%		
			HCN	KCN	K_2CO_3		HCN	KCN	K_2CO_3
	mg/10 ml pretreating mixture								
Malt	43.7	37.0 (6.61)	37.0 (5.91)	37.0 (10.12)	37.0 (8.55)	18.8	21.1	20.5	21.8
	4.86	33.3 (6.26)	33.3 (5.55)	33.3 (10.83)	33.3 (10.46)	16.8	19.4	2.75	2.00
Taka-diastase	1.38	10,000	8,000	2,900	2,000				
	0.05	12,000	10,000	2,000	1,670				

* The pH values of the pretreating mixtures are indicated in parentheses. Enzyme activities are expressed in arbitrary units per milliliter of enzyme solution.

sium carbonate was used to examine the effect of pH on the amylolytic activities of these enzymes. Concentrations of cyanide were determined by the titrimetric method of Liebig (*2*). α-Amylase activities were determined essentially by the method of Sandstedt *et al.* (*3*) with some modifications. β-Amylase activities were determined by the method of Nakamura *et al.* (*4*), the principle of which depends on the determination of the initial velocity of the liberation of reducing sugars from starch during the amylolytic reaction. Enzyme activities were expressed in arbitrary units per milliliter of the enzyme solution.

The enzyme preparations employed in this investigation included those obtained from malt, grains, sweet potatoes, and a commercial taka-diastase preparation. Ten grams each of malt, grains, and taka-diastase finely ground in a mill were treated with 90 ml of water for 1 hr at 37° with frequent shaking. The mixtures were then centrifuged and filtered. Sweet potatoes were ground on a porcelain grinder and pressed through a cotton cloth. The pressed juice was centrifuged and filtered. Purified sweet potato β-amylase was a preparation corresponding to the "purified concentrate" in the purification procedure of Balls *et al.* (*5*). Where cyanide and carbonate treatments were employed, 1 vol of 1% aqueous solutions of these inhibitors was added to 9 vol of the enzyme solutions to make 0.1% concentration of the inhibitor, and the mixture was held for 1 hr at 37°. After the treatment, the enzyme activities were determined at 37 ± 0.1°.

The results showing the effect of these treatments upon the α- and β-amylase activities of respective enzymes are given in Tables 1 and 2, respectively. Treatment of malt extracts with 0.1% KCN for 1 hr had no effect on the α-amylase activities but lowered the saccharogenic activities of these solutions. This treat-

TABLE 2

EFFECT UPON β-AMYLASE ACTIVITY OF PRETREATING VARIOUS SAMPLES WITH 0.1% SOLUTION OF HCN, KCN, OR K_2CO_3

Samples	Protein content	β-Amylase activity			
		Control	Pretreatment of the enzyme solution with 0.1%		
			HCN	KCN	K_2CO_3
	mg/10 ml pretreating mixture				
Wheat (an unknown strain)	27.1	22.2	25.3	1.30	
Wheat (Nôrin, No. 69)	18.3	17.3	17.3	0.29	0.59
Barley (Sekitori, No. 1)	36.2	27.1	26.9	10.0	18.0
	4.02	27.4	27.8	1.9	1.2
Soybean (Nôrin, No. 2)	275	53.7	53.0	52.7	58.4
	30.6	57.5	53.6	53.5	47.7
Sweet potato (Nôrin, No. 1)	17.2	494	465	429	442
	1.15	403	434	3.4	12.4
Sweet potato (Kintoki)	19.9	165	173	151	164
	1.33	166	171	13.5	7.5
Purified sweet potato β-amylase	0.77	125	157	122	116
	0.08	107	141	9.0	3.0

ment had marked decreasing effect on the α-amylase activities of taka-diastase extracts, and on the β-amylase activities of extracts from ungerminated wheat, barley, soybean, and sweet potatoes, and of solutions of purified β-amylase from sweet potato. This decreasing effect was dependent on the source of the enzyme as well as on the amount of enzyme protein in the treating mixture. Treatment of these enzyme solutions with 0.1% HCN (adjusted to pH 5.0) had no decreasing effect on either α- or β-amylase activities. Potassium carbonate in 0.1% concentration showed approximately the same degree of inhibition on these enzyme solutions as compared with 0.1% KCN. Moreover, these two reagents caused approximately the same degree of pH shift in the treated enzyme solutions.

These results support the view, in contrast to that of Roy and Underkofler, that cyanide has no inhibitory effect on either α- or β- (or saccharogenic) amylase activities of enzyme solutions used in this investigation. The apparent inhibitory effect of sodium cyanide, which Roy and Underkofler observed in their experiments, may be the result of the combined effects of pH and concentration of enzyme on the pH-heat stability properties of the enzyme solutions.

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Caloric Intake in Relation to Physique in Children[1, 2]

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It has become axiomatic today that obese children eat too much, and that linear children do not eat enough. Efforts have been made and much money has been spent by the parents of these children to change their physiques, but evidence that the results have not been satisfactory has been accumulated. On the contrary, these efforts have merely aggravated the problem and have made it even more difficult to treat. It seems pertinent, therefore, to determine to what extent the physique of the children is due to overeating and undereating, and to what extent it is due to genetic and/or other determining factors. The purpose of this study is to see whether there is a difference in caloric intake in children of different physiques. The children enrolled in the Child Growth Clinic of the Forsyth Dental Infirmary were used as subjects for this study.

Caloric intake and body build were determined for 86 subjects, selected from 350 enrolled in the aforementioned clinic, in order to obtain representatives of each physique, ranging from extremely lean to extremely fat children. Physiques were determined according to Sheldon's method. He classifies physique into three categories: (*a*) endomorphy, the stocky or obese; (*b*) mesomorphy, the muscular; and (*c*) ectomorphy, the linear or "skinny." Sheldon's contention is that all individuals have a measure of all three components, but that in the vast majority there is a degree of dominance of one of these three components which determines in which of the three categories the individual belongs (*1*). The group of 86 subjects studied consisted of 28 endomorphs, 21 mesomorphs, and 37 ectomorphs.

For each child the following was done: (*a*) height and weight were recorded; (*b*) dietary data for one week were obtained; and (*c*) the pediatrician examined each child.

The ages of these children ranged from 6 to 14 years, the mean of the group falling into the ten- to twelve-year category. Both sexes were included, although the majority were female. The physical examination of these children in the clinic revealed no evidence of pathosis.

The dietary data of these subjects for a period of one week were recorded by their mothers. The calories and amounts of proteins ingested were then calculated, and a 1-day average was computed from the 7-day data for each child.[4] The 1-day averages for each child were compared with the National Research Council standards (*2*) for a child in the appropriate age group. The results for each child were expressed as a percentage of National Research Council standards and the 1-day averages.

The mean and standard deviations of the dietary intake of each group were computed, and statistical tests for the significance of difference (*t*) between the endomorphs, mesomorphs, and ectomorphs were made.

As shown in Table 1, there are distinct differences in the ranges of caloric intake (expressed as a percentage of National Research Council requirements) of the three physique groups. The mean and standard deviations of each group are shown in Table 2.

Tests of the significance of difference (*t*) between the three groups were made, and the results are shown in Table 3. The *t* for the endomorphs versus the mesomorphs is probably significant, and the *t* for the endo-

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[4] Seasonal variations in the dietary intake were accounted for by the fact that all these children were seen either in the spring *and* fall, or in the winter *and* summer. Dietary data were obtained at each of these visits to the clinic.