Since sexual development and growth are both influenced by the endocrine balance, it appeared possible that changes produced by administration of gonadotropic and estrogenic hormones to immature animals might be reflected in the response of these animals to active immunization.

Sixteen immature male rats (3-5 days old) were injected with highly purified gonadotropic hormone from pregnant mare serum,[2] 22 with extracts of human pregnancy urine,[3] and 5 male and 6 female rats with crystalline theelin. Adult male rat pituitary glands were implanted intramuscularly in 18 immature male rats. Thirty-one male rats of the same age served as controls. All animals received a total intraperitoneal dose of 9,000 million *B. pertussis* as outlined above.

Administration of the hormones produced marked response as indicated by increase in size of the testes and seminal vesicles, and in the case of the female by increase in size of the uterus and an oestrous vaginal smear. Implantation of pituitary glands produced little if any increase in the size of testes or seminal vesicles.

The average agglutinin titers of the above groups were as follows: gonadotropic hormone from pregnant mare serum, 19; prolan, 19; theelin, 25; pituitary implants, 54; and normal controls, 36. As can be seen from this data, no significant difference existed between the agglutinin titers of normal immature rats and those receiving gonadotropic and estrogenic hormones.

8254 C

Activation of Partially Purified Pepsinogen.

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Pepsinogen can be extracted from the mucosa of the fundus part of pig stomachs by m/10 $NaHCO_3$-solution. Such extracts contain 80-90% of the total peptic activity of the mucosa (as determined after complete autolysis of the mucosa at pH 2) in the form of pepsinogen which is stable at alkaline reaction. The extract is treated

[2] Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **107**, 513.

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with Filtercel† to remove most of the mucilaginous material, which otherwise renders the extract very difficult to handle. The pepsinogen may then be gradually purified by fractional precipitation with ammonium sulphate at pH 7. (Table I.)

TABLE I.

Preparation	Yield		$Q = [P.U.]^{Hb}_{mg}$ Prot. N
	gm.*	peptic activity $[P.U.]^{Hb}$	
3000 ml $NaHCO_3$ extract from 830 gm. mucosa treated with 10% (weight) Filtercel, filtered with suction, washed with m/10 $NaHCO_3$, united filtrates (2900 ml.) 0.7 saturated with $(NH_4)_2SO_4$, after 12 hr. at 5° filtered with suction, filtrate discarded	110 g	170	0.045
Ppte. dissolved in m/10 Phosphate buffer pH 8, fractionated between 0.4 and 0.7 sat. $(NH_4)_2SO_4$ (final volume at 0.4 sat. = 1900 ml, final pH = 7.2)	—	—	0.079
Fractionation repeated between 0.46 and 0.7 sat., other conditions unchanged	35 g	90	0.105

Further fractionation was tried by means of ammonium sulphate at pH 5; this yields between 0.35 and 0.45 sat. products with Q as high as 0.145. These, however, proved to be partly activated and are therefore not discussed here. Similar results were obtained by fractionation with $MgSO_4$ at pH 5.

* Weight of moist filtercake.

The total peptic activity (pepsinogen + preformed pepsin) of the various samples was determined by the hemoglobin method[1] at pH 1.8, all pepsinogen being activated at this pH.[2, 3] Pepsinogen alone was estimated as the difference between the total activity just mentioned and the activity of samples which previously had been titrated to pH 9 in order to destroy preformed pepsin. The activity is expressed in pepsin units $[P.U.]^{Hb}$,[4] the "specific activity" (Q) being the ratio between the total number of pepsin units (pepsinogen + pepsin) and the amount of nitrogen precipitated by 4% CCl_3COOH at 80°: $\frac{[P.U.]_{ml}^{Hb}}{\text{mg Protein-N/ml}}$. The course of one preparation is given schematically in Table I.

† A commercial preparation of infusorial earth.

1 Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

2 Ege, R., and Menck-Thygesen, P., *Biochem. Z.*, 1933, **264**, 13.

3 Kleiner, I. S., and Tauber, H., *Z. physiol. Chemie,* 1933, **220**, 205; *J. Biol. Chem.*, 1934, **106**, 501.

4 Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 41.

Preparations with Q = 0.1 are but slightly colored, not mucilaginous, readily soluble in salt solutions at pH >5, stable between pH 6 and 9.

Attempts to crystallize pepsinogen have been unsuccessful so far, due either to impurities or to the fact that the isoelectric pH range, usually most suitable for the crystallization of proteins, is difficult to approach. Judging from the solubility in $(NH_4)_2SO_4$-solutions, the isoelectric pH seems to be <4 and at this pH the rate of activity is considerable.

The preparations Q = 0.1 undoubtedly contain some impurity, but in view of the fact that Q of crystalline pepsin previously has been found to be 0.2[4] and that part of the protein nitrogen of pepsinogen is split off during activation, we do not think that the amount of inert proteins present could be very high.

The rate of activation at pH 4 corresponds with the values found by Ege and Menck-Thygesen[2] in crude extracts and with a different technique. During activation there is an increase in the amount of nitrogen not precipitated by 4% CCl_3COOH at 80°. As shown in Table II, this increase runs parallel with the activation and stops at the same time. Up to this point the pepsin content increases, while the pepsinogen + pepsin remains constant. As a result Q increases and at the end of the reaction has risen to 0.2, the value for

TABLE II.

Preparation Q = 0.09, Pepsin + Pepsinogen = 0.057 $[P.U.]_{ml}^{Hb}$; pH = 4 (Acetate buffer); 35.5°.

Time	mg Prot. N/ml	Pepsinogen $[P.U.]_{ml}^{Hb}$	Pepsin $[P.U.]_{ml}^{Hb}$	% activated	% of N split off	Q
0	0.62	0.057	0	0	0	0.092
50 sec.	0.60	0.052	—	9	6	0.095
5 min.	0.53	0.042	—	26	26	0.11
13 ”	0.39	0.016	—	72	66	0.15
23 ”	0.30	0.003	—	95	92	0.19
60 ”	0.29	0.002	—	97	95	0.20
210 ”	0.27	0.001	0.056	98	100	0.21

Preparation Q = 0.15, Pepsin + Pepsinogen = 0.068 $[P.U.]_{ml}^{Hb}$ (About 70% of Pepsinogen activated during preparation); pH = 4 (Acetate buffer) 35.5°.

Time	mg Prot. N/ml	Pepsinogen $[P.U.]_{ml}^{Hb}$	Pepsin $[P.U.]_{ml}^{Hb}$	% activated	% of N split off	Q
0	0.44	0.020	0.048			0.15
40 sec.	0.43	0.018	—			0.16
2 min.	0.40	0.014	—			0.17
5 ”	0.38	0.009	—			0.18
10 ”	0.33	0.005	—			0.21
19 ”	0.32	0.004	—			0.21
25 ”	0.32	0.003	0.065			0.21

crystalline pepsin. This seems to indicate that during activation some part of the pepsinogen molecule is split off, thus transforming it into pepsin.

How this cleavage is performed, however, remains for the time being unexplained. It will be noted that the activation rate is practically constant throughout the whole reaction, in agreement with the results of Ege and Menck-Thygesen.[2] This is difficult to explain and we do not feel prepared for any discussion of the mechanism of the activation until further experimental evidence has been obtained. We should like to mention, however, that there is some slight evidence in favor of the assumption that the cleavage might be a proteolytic reaction (slow activation after infection with bacteria, *Cf.* Ege and Lundsteen,[5] Kleiner and Tauber[3]—we have had similar experiences), and we have tried, therefore, to activate pepsinogen by trypsin and papain. These attempts have been unsuccessful.

8255 C

Avitaminosis. XVII. Influence of High Fat-Containing Diets on Vitamin B_1 Requirements.*

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In a previous investigation[1] it was demonstrated that as much as 30% of fat in the diet has no sparing action on vitamin B requirements for lactation of the rat. In this study the problem has been extended to the non-lactating rat.

The following series of experiments were planned with the objective of increasing the protein as well as the fat content of the ration, at the same time keeping constant a high vitamin G content by the introduction of 15% of dehydrated autoclaved beef. The beef, purchased as round steak, was freed from all visible fat and bones and after autoclaving and drying at 100°C., contained 83% crude protein. The composition of the rations is given in Table I. It will be noted that the protein content varied from 19.98% to 33.37%, and the fat content ranged from 0 to 50%. That all the rations had a high vitamin G content became apparent after excellent growth

[5] Ege, R., and Lundsteen, E., *Biochem. Z.*, 1934, **268**, 164.

*Research paper No. 380, Journal Series, University of Arkansas, Fayetteville.

[1] Sure, B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 622.