soon after blood collection as possible, and kept at freezing temperature until tested. The samples were tested according to a recently described method capable of detecting 0.0002 μg of progesterone in 0.0006 ml of plasma and apparently specific for progesterone (*4*). In the purely qualitative tests reported on here, a positive reaction indicates a progesterone concentration greater than 0.33 μg/ml of plasma; a negative reaction signifies that, if progesterone is present, its concentration is less than 0.33 μg/ml of plasma.

The samples yielded 8 positive and 2 negative reactions (Table 1). Some samples gave a positive reaction indicating progesterone concentrations well above 0.33 μg/ml of plasma, but no attempt was made to quantitate this series. The essential point is that the positive reactions afford apparently unquestionable evidence for the occurrence of progesterone in the blood stream of the ovulating hen.

It is significant that the 2 negative reactions were given by samples taken at about the time of, or shortly following, release of ovulation-inducing hormone in so far as this has been estimated by injection procedures (*3*) and hypophysectomy (*6*). The occurrence of low values at so critical a phase of the ovulatory cycle may be taken as substantial evidence for ovarian, and most likely follicular, origin of progesterone, an inference in good accord with recent views of the mammalian follicle (*1*).

TABLE 1

PROGESTERONE REACTION OF PLASMA SAMPLES TAKEN WITH REFERENCE TO ESTIMATED TIME OF SECOND OVULATION OF THE CLUTCH

Sample No.	Hen	Time of blood withdrawal referred to 2nd ovulation	Progesterone reaction*
1	A	11.5 hrs before	+
2	A	9.5 " "	+
3	A	8.0 " "	+
4	A	7.0 " "	+
5	A	6.0 " "	–
6	B	3.7 " "	–
7	B	1.7 " "	+
8	A	0.5 " after	+
9	C	4.5 " "	+
10	C	8.5 " "	+

* A plus sign indicates a progesterone concentration of more than 0.33 μg/ml of plasma; a minus sign, a concentration of less than 0.33 μg/ml.

The observations which encouraged undertaking the tests reported here will be accounted for elsewhere in some detail. Further tests are contemplated to determine the site or sites of origin of progesterone in the bird, as well as exact concentrations of progesterone throughout the ovulatory cycle.

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Phosphorylase in Guard Cells

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In a previous report (*5*) a histochemical method was described for the detection of phosphorylase in plant cells and tissues. Briefly, the method consists of incubating free-hand sections of plant parts, previously freed of starch, in a buffered medium containing glucose-1-phosphate and subsequent staining with iodine in potassium iodide. Using this method, a number of experiments have been made. The results will be published elsewhere. The present note gives a brief summary of observations on plant epidermis.

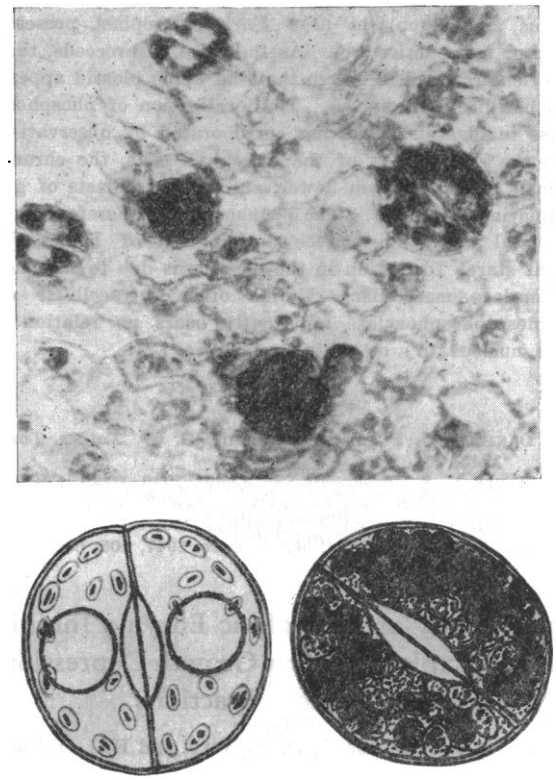

FIG. 1

Leaves of tobacco and broad bean were starved in darkness to free them of stored starch. The epidermis was stripped off and treated with glucose-1-phosphate as described above.

Very intense starch formation is found in the guard cells after 4–5 hrs of incubation, indicating the presence of strong phosphorylase activity. Little or no reaction is shown by the epidermal cells (see Fig. 1). Incubation with glucose instead of glucose-1-phosphate with or without the addition of magnesium salts and adenosinetriphosphate fails to give rise to starch even after 24 hrs. The result indicates that the guard cells

possess no detectable phosphorylating mechanism, although they show some phosphatase activity (*4*). The high concentration of phosphorylase in the guard cells suggests that the enzyme may play a part in the osmotic changes of the cells and therefore in the movement of stomata. Experiments along this line are now in progress.

Closer observations reveal that the phosphorylase activity is exclusively localized in the chloroplasts. It is especially clear in cells which have been incubated for but a short time before too much starch has been accumulated (Fig. 1). No activity is found in the nucleus and cytoplasm (*1*). Each chloroplast possesses one or more active loci. As the reaction proceeds, these loci enlarge and merge so that the whole plastid appears to be filled with starch. The localization of phosphorylase in plastids is further corroborated by observations on the chloroplasts of the mesophyll cells, the chromoplasts of Tropaeolum flower, and the leucoplasts of germinating seeds (*4*). The phenomenon is therefore quite general and confirms the anticipation of Hanes (*2*) that starch formation in the plastids is due to the phosphorylase mechanism. Unlike other intracellular enzymes, phosphorylase apparently bears no relation to the nucleus (*1*) or the mitochondria (*3*).

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Inadequacy of Proteolytic Enzyme Inhibition as Explanation for Growth Depression by Lima Bean Protein Fractions

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Discovery of the presence of trypsin inhibitors in soybeans (*3, 7*) and other legumes (*2*) and the ability of concentrates of these factors to inhibit growth in rats (*11*) and chicks (*8*) have stimulated interest in the mechanism involved in the poor nutritional values of the proteins of raw legumes.

An obvious hypothesis is that these bean fractions exert growth-inhibiting effect through their ability to inhibit normal enzymatic protein hydrolysis in the intestinal tract. This report concerns experiments with hydrolyzed proteins which have given results contradictory to that hypothesis.

Desikachar and De (*6*) have recently reported that active extracts of the soybean trypsin inhibitor had a depressing effect on the biological value of papain-digested soybean meal essentially equal to their effect on undigested meal. The two values reported, 45.9 and 44.7, respectively, were quite low, and presumably much lower than a value for undigested soybean meal in the absence of the inhibitor fraction would have been if data on such a positive control had been obtained. Although these data suggest that the low biological value of raw legumes is not due primarily to inhibition of enzymatic digestion, it must be pointed out that several studies (*1, 4, 9, 12*) have shown that papain, at a maximum, hydrolyzes only one-half to two-thirds of the peptide bonds in casein and other proteins. Also, solubility of 91.6% of the nitrogen of the papain digest in 7% trichloroacetic acid, used by Desikachar and De to estimate completeness of hydrolysis by papain, does not preclude the existence of some soluble peptides (*1, 5*).

Hence, the possibility has remained that the low biological value found for the papain-digested meal was due to inhibition of hydrolysis of residual bonds. As a part of our continuing study of the growth inhibitors in legumes, we have carried out experiments that eliminate this possibility in so far as trypsin-inhibiting activity obtained from lima beans is concerned.

Lima bean protein fractions that contained high *in vitro* trypsin-inhibiting activity and also high growth-inhibiting activity in normal diets were fed to rats on diets containing completely acid-hydrolyzed casein as the sole source of nitrogen. In addition to nitrogen-contributing components, the diets contained, in per cent: cottonseed oil, 5; U.S.P. cod-liver oil, 2; salt mixture (McCollum's No. 185 plus trace elements), 4; dried liver extract (Wilson), 0.4; protein-free yeast extract equivalent to 2% yeast, and, as mg%, α-tocopherol, 5; choline chloride, 150; inositol, 100; thiamin hydrochloride, 0.5; riboflavin, 1.0; pyridoxin, 0.5; nicotinic acid, 1.0; calcium pantothenate, 2.5; *p*-aminobenzoic acid, 7.5; 2 methyl-1,4-naphthoquinone, 0.2; biotin, 0.01; and sufficient equal-weight mixture of corn starch and cane sugar to make 100%. All diets contained 15/6.25% = 2.4% nitrogen, made up in diets 1 to 5 from 0.4% *dl*-tryptophan, 0.6% *l*-cystine, and 20% casein hydrolysate and in diets 6 and 7, from commercial casein, plus, in both cases, the nitrogen contributed by the lima bean fraction when present. The lima bean fraction was added to the diet without an equivalent deduction in amount of casein-hydrolysate nitrogen in order to eliminate the possibility that the inhibiting effect would be even partly due to a lowered concentration of available amino acid or protein nitrogen in the diet.

Casein hydrolysates were prepared by autoclaving commercial casein with four parts of 9N H_2SO_4 at 15 lbs of pressure for 18 hrs. The sulfuric acid was removed with barium hydroxide to a pH of 4 to 5, and the resultant filtrate was concentrated *in vacuo* and dried from the frozen state. The completeness of hydrolysis was verified by amino nitrogen analyses, which indicated 71–74% of total nitrogen present as amino nitrogen (*13*).

A modification of Kunitz' (*10*) method of isolating the

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