hundred castrated animals over a period of 2 years, has indicated that the necessary smears for practical use should be 48, 56, 72, 80, and 96 hours after the animal receives its injection. If the animals are injected on Monday A. M. this arrangement calls for smears to be recorded Wednesday A. M. and P. M., Thursday A. M. and P. M., and Friday A. M. If a full estrus reaction is not found during these intervals the result is negative. When estrone is administered the smears taken at the same intervals should also reveal a full estrus reaction or the animal should not be considered controlled. The route of injection must be subcutaneous, both with the standard and the unknown; injections must be single in number and always in the same solvent, olive oil. The use of fewer than 20 animals as stipulated for each test dose in the Report of the Second Conference on the Standardization of Sex Hormones[11] can be satisfactory only if these animals are individually examined and suitably controlled.

These studies do not indicate that different groups of mice have a definite constant threshold of reactivity to estrone but rather that by first overstimulation and then persistent adequate stimulation at regular intervals mice can be brought into, and then maintained in, a state of maximal reactivity to a rather constant minimal dose of estrone.

9141 P

Distribution of Phosphorus in the Starch Granule.

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Gelatinized root-starch granules possess a peripheral membrane containing phosphoric-acid ester. This has led some investigators to infer that the natural ungelatinized granule also possesses such a membrane. If this be so, it follows that the P_2O_5 percentage in the small granules of a given sample should be greater than in the large granules, provided the thickness of the hypothetical membrane of the two sorts of granules is about the same.

There are two pertinent observations in the literature. Fernbach[1] found the small-sized granules to contain decidedly more P_2O_5 than

[11] *Quart. Bull. Health Organization League Nations*, 1935, **4**, 618.

[1] Fernbach, A., *Compt. rend.*, 1904, **138**, 428.

the large-sized ones in the same sample of potato starch. He concluded, however, that the granule consists of a nucleus rich in phosphorus upon which are superimposed layers of phosphorus-free material. Kavcic[2] determined the P_2O_5 content of starch from 4 potato varieties with granules of different average diameters. While his figures indicate a tendency for the P_2O_5 percentage to increase as the average granule size decreases, the differences are not as great as they should be considering that the mass of a solid sphere increases as the diameter increases far more than the surface area. The interpretation of such data as these of Kavcic is difficult without knowledge of the frequency distribution of granule sizes in each sample. Another difficulty is the possibility that the P_2O_5 content of starches varies with the variety or the conditions of growth independently of granule size. Mangels[3] has reported such variations for wheat, though he did not correlate them with granule size.

All these difficulties may be avoided by separating each starch sample to be analyzed into fractions of different granule size, and analyzing each fraction separately. The writer had a sample of cassava (tapioca, manioc) starch so separated in the metallurgical laboratory of the university by air flotation in an air classifier. E. Hines analyzed the several fractions by Embden's method for phosphorus. The several fractions had very much the same P_2O_5 content.

It is evident, therefore, that there are starches in which phosphorus content and granule size are not directly correlated. This conclusion is in harmony with the view of Taylor and Schoch[4] that phosphorus is distributed quite irregularly through the granule. Indeed, they believe that the chain molecules of starch "may vary over a wide range with regard to the amount of phosphate held to them, giving rise to a kind of heterogeneity even though all these fractions unlike those from the cereal starches are soluble." Maquenne and Roux[5] also believed that the phosphoric-acid ester of starch (α-amylose or amylopectin) is intimately mixed with the other constituents of the granule.

With the evidence at hand, we are not warranted in inferring that because gelatinized granules possess a peripheral membrane containing phosphoric-acid ester natural ungelatinized granules also possess such a membrane. Indeed, there is no satisfactory evidence that natural granules possess any membrane at all, except remnants of

[2] Kavcic, J., *Kolloidchem. Beihefte*, 1930, **30**, 406.

[3] Mangels, C. E., *Cereal Chem.*, 1934, **9**, 571.

[4] Taylor, T. C., and Schoch, T. J., *J. Am. Chem. Soc.*, 1933, **55**, 4248.

[5] Maquenne, L., and Roux, E., *Comp. rend.*, 1905, **140**, 1303.

the plastid in which they are formed and these are usually removed in the process of purification. There is, in fact, evidence to the contrary. If α-amylose or amylopectin were localized at the periphery, one would expect those natural granules to have the thickest and strongest membranes that have the greatest phosphoric-acid content. One would expect such granules when boiled to hold together better than sorts with a lesser phosphoric-acid content. As a matter of fact, there is no such relationship between phosphoric-acid content and mechanical resistance to boiling. Potato starch with a fairly high phosphoric-acid content disintegrates rapidly, whereas tapioca starch, also a root starch, but with a much lesser phosphoric-acid content does not. The observations of Lynst-Zwikker,[6] which the writer has been able to confirm, indicate that the membrane of gelatinized granules is an artifact formed during gelatinization by the accumulation of the less soluble materials at the periphery of the granule.

9142 P

Differentiation between Vitamin B_4 Deficiency and "Encephalomalacia" in Growing Chicks.

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We[1] described a deficiency disease in growing White Leghorn chicks which was corrected by concentrates of Reader's vitamin B_4. The main symptoms described were lack of growth and incoordination which resulted in extreme difficulty in locomotion. Accompanying the lack of coordination of the muscles and disturbed gait was a tendency of the bird to fall upon its side with the legs in tension and pulled against the abdomen. Examination of the brains of a number of the deficient birds revealed gross lesions in the cerebrum and degeneration which could be observed histologically in both the cerebrum and cerebellum. At that time we stated that the condition encountered was probably the same as that observed in chicks by Pappenheimer and Goettsch[2] and designated by them as

[6] Lynst-Zwikker, J. J., *Rec. trav. bot. néerlandaises*, 1921, **18**, 1.

[1] Keenan, J. A., Kline, O. L., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *J. Biol. Chem.*, 1933, **103**, 671.

[2] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.