In several cases the anterior limbs budded out and differentiated in a normal manner, showing all the digits completely formed; such larvæ would swim about in a lively manner when they were stimulated. In fact the posterior pieces of these embryos developed much beyond the stage at which normal lavræ begin to take food, but whether lack of food precluded them from developing farther cannot as yet be stated.

A definite circulatory system is not necessary for the development of legs, gill filaments, the growth of the tail or the differentiation of internal organs up to quite a late period of larval life. The absence of the thyroid, thymus and pituitary body seems to produce no marked changes up to the period in which the legs and gills are well developed.

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On "racemized" casein.[1]

By CARL L. A. SCHMIDT.

[*From the Rudolph Spreckels Physiological Laboratory and the Hearst Laboratory of Pathology and Bacteriology of the University of California.*]

The remarkable observation made by Dakin and Dudley[2] that "racemized" casein is not attacked by pepsin, trypsin, or erepsin, is apparently excreted unchanged when injected subcutaneously or given by mouth to a dog, and is unaffected by bacteria, would indicate that the chemical structure of casein had been decidedly altered during the process of "racemization." Dakin[3] suggests that the process is a tautomeric change of the keto-enol type in the manner $> CH—CO—NH— \rightleftarrows > C = C—OH—NH—$ and that in the case of "racemized" casein this change must be complete for all the groups in the protein molecule, otherwise a point of attack for enzymes would be afforded and a partial splitting might occur. If the ketol-enol conversion be complete, the apparent discrepancy pointed out by Kober that all racemic peptides are attacked by erepsin would not necessarily be a real one, since

[1] Aided by a grant from the George Williams Hooper Foundation for Medical Research.

[2] Dakin, H. D. and Dudley, H. W., *J. Biol. Chem.*, 1913, 15, 271.

[3] Dakin, H. D., *J. Biol. Chem.*, 1912–1913, 13, 357.

the tautomeric change suggested by Dakin is not racemization in the ordinary meaning of the term. The discrepancy as to the number of end amino groups in casein, as indicated by the optically active amino acids found by Dakin, is as Kober[1] shows, fatal to Dakin's theory.

Anticipating the probability that a substance which is so changed as to be unaffected by enzymes and bacteria might also be changed in its antigenic properties, Ten Broeck,[2] at Dakin's suggestion, carried out experiments with "racemized" egg albumin and found that the substance was non-antigenic as shown by the anaphylaxis, precipitin and fixation tests. Underhill and Hendrix,[3] on the basis of fall of blood pressure and retardation of blood clotting, find that "racemized" casein, egg-albumin and zein are non-toxic, but yield toxic products on partial hydrolysis with acid. The "racemic" proteoses, with the curious exception of zeose, are likewise toxic. It appears, then, that the "racemic" substances are not entirely without action on the body when introduced parenterally.

The "racemization" of proteins appears to throw some light on the relationship of structure to antigenic property. Based on "racemization" experiments, the work of Dudley and Woodman[4] would indicate that casein from the sheep differs from casein obtained from cow's milk.

In connection with other work being done on the relationship of protein structure and antigenic property, it appeared to the writer of importance to fill in the gap which in the particular case of casein is still missing, and to determine whether racemized casein is likewise non-antigenic. "Racemic" casein was prepared essentially according to Dakin's method, except that for the purpose of drying the product readily, it was washed with alcohol and ether which removed the larger portion of the caseose with which it is usually contaminated. In preparing solutions of both casein and the racemized product, sufficient alkali was added to make the solution neutral to litmus. The rabbits received injec-

[1] Kober, P. A., *J. Biol. Chem.*, 1915, 22, 433.

[2] Ten Broeck, C., *J. Biol. Chem.*, 1914, 17, 369.

[3] Underhill, F. P. and Hendrix, B. M., *J. Biol. Chem.*, 1915, 22, 453.

[4] Dudley, H. W. and Woodman, H. E., *Biochem. J.*, 1915, 9. 97.

tions over a period of a month with the substances and in the manner and dosage as follows:

"Racemized" casein, No. 869—One 100 mg. dose intraperitoneally and seven 100 mg. doses intravenously.

No. 670—Five 100 mg. doses intraperitoneally and three 100 mg. doses intravenously.

Nos. 849 and 850—Five 100 mg. doses intraperitoneally and four 100 mg. doses intravenously.

No. 871—Previously immunized with casein and high titer serum obtained.

Eight days after the last injection the animals were bled and fixation experiments carried out in the usual manner, using one fourth of the minimum inhibiting dose of antigen and 1.5 units of alexin. The serum of animals immunized with casein gave positive tests with quantities of serum less than 0.02 cc. (titration not carried to limit); the sera of animals injected with racemized casein were negative in doses which themselves were not inhibitive on omission of the antigen. Similar tests using the heterologous antigen showed no fixation, showing that "racemized" casein is not identical with casein.

The observation made by Robertson[1] that when a current is passed through a solution of casein a precipitation of casein occurs on the anode, was found to be also true for the racemized product. It must therefore be dissociated. But, as pointed out to me by Dr. Robertson, if Dakin's theory of the ketol-enol conversion be true, we would be compelled to assume that the "racemic" protein dissociates in a different manner than the now generally accepted theory proposed some years ago by Robertson.[2] According to his theory the dissociation of the protein salts with inorganic bases and acids is accomplished in the manner

$$\text{—COH} = \text{N—} + \text{N}\dot{\text{a}} \qquad \text{O}\acute{\text{H}} \rightarrow \text{—CO}\overset{+}{\text{N}}\dot{\text{a}} + \text{N}(\text{H}'')(\text{OH})\text{—}.$$

If the "racemization" of casein is due to the oscillation of a labile hydrogen atom attached to the carbon atom, we must either assume, if the substance dissociates in the usual manner, that hydro-

[1] Robertson, T. B., *J. Phys. Chem.*, 1911, 15, 179.

[2] Robertson, T. B., *J. Phys. Chem.*, 1911, 15, 521.

gen is split off, or if hydrogen is retained, we must have two protein ions each having only a single latent valency capable of neutralizing a unit charge as the following will indicate:

$$= C(OH)—NH— + N\dot{a} \qquad O\acute{H} \rightarrow = C\overset{+}{O}Na + \begin{array}{c} H' \quad H \\ \diagdown \quad \diagup \\ N— \\ | \\ OH \end{array}.$$

Such a case has not as yet been shown, though the possibility may exist. It is also possible that a change other than the ketol-enol tautomerism takes place.