transaminase[10]), about 50% decrease of threonine plus serine (substrate of serine dehydrase) and approximately 25% reduction in the level of total free amino acids. There was no indication that the enzyme elevation caused by starvation was associated with elevation of their substrates in the liver. The above findings, then, suggest that starvation initiated the elevation of the two enzymes in the liver by a mechanism independent of either glucocorticoid or substrate induction.

The reason for the adaptive increases of the hepatic transaminase and the dehydrase in starvation is not clear, but the physiological relevance of the changes is understandable. The critical need for energy in starvation is ultimately contributed by tissue protein catabolism. The elevation of specific amino acid catabolic enzymes in such a situation is an appropriate adaptation towards homeostasis. Accordingly when an external energy source, D-glucose, was provided in gram quantity to the rats with the onset of starvation, the enzyme elevation, as shown in the Table, was either abolished or suppressed. An inhibitor in the enzymes of the starved rats given glucose could not be detected. The sugar or its metabolites (glucose-6-phosphate, glucose-1-phosphate, lactate) when added in vitro in millimolar amounts did not inhibit the enzyme activities. Also the administration of the sugar to fed rats did not lower the basal transaminase and dehydrase activities of the liver. Such an in vivo inhibitory effect of injected glucose was previously reported[11] with the starvation-induced adaptive increase of cysteine desulfhydrase activity in the chick liver. It is of interest that similar inhibition by glucose of the adaptive increases of a microsomal enzyme, liver dimethylaminoazobenze reductase induced by starvation[12] and liver threonine dehydrase and ornithine transaminase by casein feeding[13] has also been reported in recent publications.

Résumé. Les activités de la tyrosine transaminase et de la sérine déhydrase sont augmentées dans le foie de rats normaux ou surrénalectomisés soumis au jeûne. Ces augmentations, qui sont supprimées par la puromycine ou le glucose, ne sont dues ni à une sécrétion d'hormones surrénaliennes ni à une accumulation des substrats des enzymes.

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Role of Heavy Water as Solvent in the Antigen-Antibody Precipitin Reaction

Mice drinking 30% heavy water (D_2O) have been observed to manifest depressed responsiveness to antigenic stimuli[1]. The mechanism of this suppressive action, however, has not been elucidated. In this regard, experiments have been designed to distinguish between the effects of D_2O acting as solvent and source of readily exchangeable hydrogen isotope and D_2O acting as a source of non-exchangeable isotope incorporated by synthesis into C–D bonds. The present report is a description of such an experiment carried out in vitro, in which the effects of D_2O as a solvent system for the quantitative precipitin reaction were studied. To maximize such effects, the level of D_2O employed (50%) was twice that extant in the body fluids of mice on a 30% D_2O regimen[2].

Serum from a normal albino rabbit immunized by a single subcutaneous injection of 2.0 ml complete Freund's adjuvant containing 10 mg bovine serum albumin was collected by heart puncture on the 50th day post-immunization. Serum was filtered and inactivated at 56 °C for 30 min. One volume was then added to an equal volume of isotonic 99.7% D_2O saline. As a control, a sample of the same serum was similarly diluted with isotonic saline and both samples incubated at room temperature for 74 h. At the end of the incubation period, pH measurements were made on both samples. Optical densities were determined in the Beckman DB spectrophotometer between 240 and 320 nm on 1:100 dilutions of each sample, using the corresponding aqueous or deuterated saline as diluent. Antigen-antibody reactions using $^1/_2$ ml of the 1:1 diluted antisera were carried out in an isotonic pH 7.4 saline borate buffer, in a volume of 2.5 ml. These systems were maintained aqueous or 50% with respect to D_2O to correspond respectively with the aqueous or deuterated antisera employed. After addition of antigen, samples were incubated for 1 h at 37 °C and refrigerated at 4 °C for 7 days. Specific precipitates in both sets were then washed twice with 3 ml of 0.15 *M* NaCl near 0 °C, digested and nitrogen analyses carried out in duplicate for each point by a micro-Kjeldahl method.

As seen in the Figure, the quantitative precipitin behavior of normal rabbit antibody in 50% D_2O was observed to coincide with that of the control in the antibody excess zone, although an increased formation of precipitate was noted in the region of antigen excess. The pH and UV-spectral characteristics of the D_2O and H_2O dissolved antisera employed in this assay were found to be similar. Normal precipitin behavior in the antibody excess zone indicated that no loss of combining ability had occurred. Increased precipitate observed in the antigen excess zone might be ascribed to diminished solubility of normally soluble antigen-antibody complexes, since the solubility of a variety of polar compounds in heavy water has been shown to be markedly less than in

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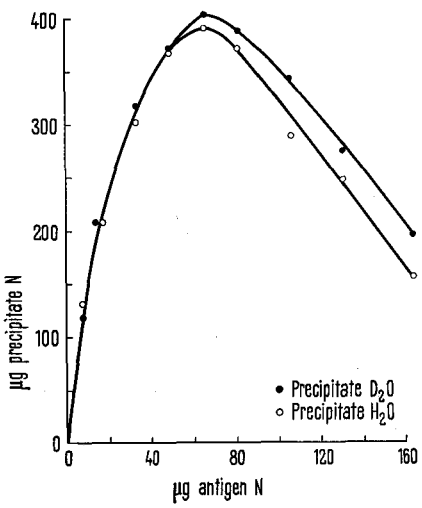
ordinary water[3]. Non-specific aggregation of antibody globulin in heavy water did not appear to contribute to this increased antigen excess zone precipitation. It was previously demonstrated by MORTON[4] that 7S globulin aggregated by X-irradiation, and likewise native macroglobulin, manifested increased UV absorption and an increased 250/280 optical density ratio when compared to equal weights of native 7S material. In the present experiment, comparable alterations in UV spectra were not observed, suggesting minimal, if any, aggregation.



Formation of immunological precipitates in buffered systems containing H_2O or 50% D_2O.

The partial replacement of amide, plus side chain N–H, O–H, and S–H hydrogens of normal antibody by deuterium as described here would be analogous only in part to the molecule formed in the deuterated animal, since in the latter a substantial proportion of non-exchangeable hydrogens would also be replaced as a result of biosynthetic processes[2]. Thus the possibility remains that the antibody molecule as synthesized in the deuterated animal may possess altered chemical and biological properties[5].

Zusammenfassung. Wenn die Lösung zu 50% aus schwerem Wasser bestand, blieb die Bildung von Antigen-Antikörperpräcipitat im Bereich des Antikörperüberschusses unverändert. Dies zeigt, dass die Substitution mit Deuterium die spezifischen Bindungseigenschaften nicht verändert. Dagegen fand sich eine erhöhte Niederschlagsmenge im Bereich des Antigenüberschusses, was auf eine Herabsetzung der Löslichkeit des normalerweise löslichen Antigen-Antikörperkomplexes hinweist.

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On the Digestive Enzymes and the Cytology of Midgut of *Leogryllus bimaculatus* Sauss.

The various cell extrusions, often observed along midgut epithelia of insects as cytoplasmic globules, separated cell tips, bursting and extruding cells, some or other of which have been regarded by a large number of previous workers, such as VAN GEHUCHTEN[1], GRESSON[2], HODGE[3] and SAKSENA[4], to be evidence of secretory activity, have been considered by some other workers, such as HENSON[5], WOODRUFF[6], DAY and POWNING[7] and KHAN and FORD[8], to represent cellular degeneration. The present author[9–11], on the basis of his investigation on structure and activity of the epithelial cells in midgut and hepatic caeca of certain insects under normal and induced pathogenic conditions, had also earlier asserted that these cell extrusions appear to represent cellular degeneration.

The present investigations have been carried out to see if there is any relationship between the production of digestive enzymes and extrusion of the so-called secretory vesicles etc. in midgut of *Leogryllus bimaculatus* Sauss.

Normally feeding insects and specimens starved for long periods, from a stock of *Leogryllus bimaculatus* Sauss. maintained in the laboratory on a diet of bread, liver and sugar, were dissected, part of their midgut was fixed by Yao-Nan and Mann-Kopsch techniques and 4–5 μ thick sections were obtained, while the rest of the parts of midgut were employed for qualitative enzyme estimations.

Experiments conducted to determine the enzymes present in normal feeding specimens reveal the presence of amylase (Potassium iodide-iodine test), invertase (Barfoed test), maltase (Barfoed test), lipase (bromo-thymol blue and milk test), lactase (Barfoed test) and protease (egg albumin test). These enzymes show gradually weaker reactions in starved specimens and are entirely absent in specimens starved for long periods. Histological preparations from normally feeding specimens show that in the

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