to rapid metabolism of this compound in the liver[10,11] and excretion in the kidney[12].

The significance of this pathway in the overall metabolism of this trypanosome is presently obscure. The initial transamination of tryptophan could serve to increase the intracellular concentration of glutamate, thereby stimulating conversion of pyruvate to alanine by alanine aminotransferase, an enzyme present in high levels in this parasite (unpublished data). The result, in addition to detoxification of intracellular pyruvate, might be an increased rate of glycolysis due to removal of the end-product[13].

Résumé. Les trypanosomes ont converti les substrats L-tryptophan et DL-5-hydroxytryptophan en métabolites tryptophol (indole-3-éthanol) et 5-hydroxytryptophol, deux composés qui produisent le sommeil chez la souris et le poussin. Les effets possibles de ces composés soporifiques chez un homme infecté par ce parasite et leur rôle dans le métabolisme du parasite, sont discutés.

H. H. Stibbs and J. R. Seed

Laboratory of Parasitology, Department of Biology, Tulane University, New Orleans (Louisiana 70118, USA)
12 May 1973.

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COGITATIONES

The Mechanism of Specific Precipitation: Another Look

The 'lattice' (= framework = alternation) theory of specific precipitation, proposed by Marrack[1], was later adopted by Heidelberger and Kendall[2,3], by Pauling[4], and by various other workers in the field. Briefly, it supposed that (assumed) multivalent antibody united with multivalent antigen, each such union involving only a single combining group of each reagent, that the bimolecular compound thus formed then united with another molecule of antigen or antibody (or with an antibody-antigen compound already formed), and that this process continued until the resulting aggregates were so large that they perforce separated out of the solution as a precipitate. The idea had a refreshing mechanistic simplicity, and to workers trained mainly in chemistry, and not over-familiar with the earlier literature of immunology, the theory seemed almost self-evident, as indeed some of its proponents claimed it was. This lattice theory, in spite of some opposition, has become the generally accepted theory.

Nevertheless, it was clear from the beginning that the lattice theory was not an adequate explanation of all the known facts. Rather surprisingly for a theory suggested by chemists, it took no account of solubility. Heidelberger and Kendall[5] explicitly stated '...aggregation would occur regardless of the affinity of the groupings for water.'

But, contrary to this idea, it was already known that affinity of the groupings for water, i.e., solubility, did play a role. For example, it had been found that precipitates made with protein and horse anti-protein antibodies were soluble in excess antibody, whereas such precipitates made with rabbit antibody were not. Clearly a solubility effect. Boyd and Purnell[6] studied the precipitating behavior of the different kinds of antibody in detail.

The lattice theory demanded that antigen be multivalent, which was never in much doubt, and which subsequent investigation has amply confirmed (cf. [7]), and assumed antibody also to be multivalent, a more doubtful point. Later work has in fact not confirmed it, but it does seem that most antibody is divalent (cf. [7]), and divalent antibody can be visualized as forming 'lattices' with multivalent antigens or haptens.

The lowest valence that can be called multivalent in this sense is three, and in fact some trivalent haptens have been observed to precipitate with appropriate (divalent) antibody. But with divalent haptens and divalent antibodies lattice formation is hard to visualize. Rather awkwardly, some divalent haptens have been observed to precipitate. However, some of these haptens are probably aggregated in solution[8], and thus become effectively multivalent, which may be an explanation.

It was suggested that divalent haptens, even when they did not precipitate, should form long ...hapten-antibody-hapten-antibody... chains. This should confer on the mixture a pronounced birefringence of flow, but a careful examination of such mixtures by Hooker and Boyd[9] did not reveal any such birefringence. In fact, later studies by Epstein, Doty and Boyd[10], using the light scattering technique, indicated that the chains formed in their mixtures were short. They did not precipitate.

It was obvious that the behaviour of di- and multivalent haptens when mixed with antibody would be a critical test of the lattice theory. Boyd[11] studied the reactivity of 34 different haptens containing from 1 to 6 specifically reactive groups, and found that 6 divalent haptens, 4 trivalent haptens, and 1 hexavalent hapten failed to precipitate. He was able to connect these observations plausibly with the solubility of the haptens. He concluded that the possibility of lattice formation is by no means sufficient to ensure that a hapten will precipitate.

Boyd[11] stated, 'As an explanation of precipitation, it would seem that neither the alternation nor the Bordet theory is adequate. The alternation theory seems to be simply incorrect, and the Bordet theory too vague to

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account for the very definite facts presented here. For the theory suggested above... namely, that the precipitation is due to the lowering of solubility by neutralization of polar groups of antibody and hapten (or antigen), and concommitant steric hindrance of other polar groups of neighborning molecules in the complex, I wish to propose the name, occlusion theory.'

PAULING never referred to the occlusion theory; he and co-workers[12] later found that 20 haptens, each containing two or more combining groups per molecule, always precipitated with each of his 4 antisera. PAULING el al.[12] dismissed BOYD's contrary results by saying, '...we consider it likely that his experiments were carried out under conditions unfavorable to precipitation – his antisera may have been too weak, or his antigens may have contained monohaptenic impurities.' They evidently did not compare the reported strength of BOYD's sera with their own, or they would have noticed that BOYD's strongest serum contained more antibody per ml than did the weakest of their sera, which they nevertheless found to precipitate all their haptens. As to the other objections; I stick to my guns; my compounds were pure, and my conditions ideal for precipitation.

As already said, the lattice theory was eventually virtually universally accepted. But there are indications that opinion is beginning to change. MARRACK[13], always one of the clearest and most objective thinkers in this field, expressed himself in 1961 as no longer entirely entirely satisfied with his own lattice theory, and stated that '...we are now back to BOYD's occlusion theory.'

The there are some recent experiments that bear importantly on the question. It was always obvious that if specific precipitation should ever be observed with a *univalent* hapten, the lattice theory would have to be modified or abandoned. For it is impossible to imagine lattice formation with antibody and a molecularly dispersed univalent hapten. When the theory was proposed, however, this had never been observed, even in the extensive inhibition studies carried out by LANDSTEINER and his many followers, including myself. But at last the unexpected seems to have happened. SPRINGER and DESAI[14] report that the 7S globulin of eel serum that possesses specific blood group anti-H (0) activity precipitates specifically with either of 2 monohaptenic monosaccharides, viz., 3-*O*-Methyl-D-fucose and 3-*O*-methyl-D-galactose. The possible objection that these monosaccharides might be aggregated in solution was disposed of by vapor pressure osmometry and freezing point depression measurements. With admirable restraint SPRINGER and DESAI remark, 'It is difficult to reconcile these findings with the lattice theory of immune precipitation...'.

It seems possible that the whole question of the mechanism of specific precipitation ought to be reconsidered.

Zusammenfassung. Neue Beweise für eine Theorie der Präzipitationsreaktion (BOYD's «occlusion-theory»).

W. C. BOYD[15]

Department of Immunochemistry, Boston University, School of Medicine, Boston 18 (Massachusetts, USA), 2 July 1973.

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STUDIORUM PROGRESSUS

Morphological and Enzymehistochemical Changes in the Interscapular Adipose Tissue of Adult Guinea-Pigs During Prolonged Exposure to Cold

In mammals the function of brown aidpose tissue has been described as thermogenic, both in the infant or when the animal is exposed to cold[1].

In the newborn guinea-pig, the interscapular adipose tissue is typical brown fat with multilocular cells and has remarkable thermogenic ability. However, after 3 or 4 weeks, thermogenesis becomes impaired as the multilocular fat cells change to unilocular[2]. This cytological change is associated with a disappearance of the histochemical reactions of cytochrome oxidase, β-OH butyrate and succinate dehydrogenase, and monoamino oxidase from the fat cells. Thus, after 3 weeks, the morphology and the enzyme pattern of interscapular fat cells resembles more that of the white fat cell than of the brown fat cell[3]. Thus, the guinea-pig differs from the rat which retains some brown adipose tissue throughout life, irrespective of age or ambient temperature.

However, thermogenesis in the unilocular interscapular adipose tissue can be reactivated by exposing the guinea-pigs to prolonged cold. This effect is more pronounced in young animals than in older ones. When thermogenesis is increased, at least part of the fat cells in the interscapular fat pad are multilocular, being scattered among the unilocular fat cells[4].

Based on the above observation, adult guinea-pigs raised at room temperature were exposed to prolonged cold stress and the histochemical reactions of some oxidative enzyme were studied in the interscapular fat in association with morphological changes. Further, the approach was expanded to include electrophoretic characterizations of two of the enzymes which display strong histochemical reactions in both types of fat cells. We hypothesized that if the reappearing multilocular fat cells were indeed brown fat, then some of the enzymes of brown adipose tissue that are normally associated with a high potential for oxidative metabolism should be directly verifiable by histochemistry.

Material and methods. Before the main experiments, a pilot test was made with 2 guinea-pigs at 5 °C $\pm$ 2 °C for 1 month and 1 control at 24 °C $\pm$ 2 °C. After acclimatization, samples from the interscapular adipose tissue (IAT) were excised and the wounds were left to heal for 1 month. These guinea-pigs were then included in the first longer acclimatization test. The samples showed no changes in morphology during the 1 month's cold acclimatization

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