nancy sera was not antagonised by pretreating the test animals with rather large doses of corticosteroids, salicylate or aminopyrine. This refractoriness to known anti-inflammatory agents is all the more astonishing when compared with the extreme lability of the necrotising action. Thus, mere heating of active sera for as little as 5 min at 56°C, or mixing active sera with equal volumes of knee or pleural exudate, normal serum or Tyrode's solution abolished the necrotising effect either completely or to a considerable extent. In some cases knee exudate was very active in respect of its 'neutralising' effect in that as little as 2 parts of exudate added to 8 parts of a +++ serum were able to abolish the necrotising capacity of the serum. On the other hand, no such loss was observed when equal volumes of two active rheumatoid sera or of rheumatoid and malignancy serum were mixed.

In accordance with BOAKE and LOVELL[4], it was also observed that the factor causing skin necrosis is not identical with Forssman's antibody or complement, nor were attempts to produce skin lesions with precipitates prepared in the cold according to SVARTZ[7,8] successful. No correlation was found between serological tests and the guinea-pig skin activity, the latex test for example being negative in a considerable number of cases with sera producing +++ lesions. The necrotising activity seemed to run to some degree parallel with the erythrocyte sedimentation rate, an observation also made by KLEMPERER et al.[6]. On the whole, the guinea-pig skin test reflected the activity of a given disease far more than the stage to which it had progressed. Furthermore, no appreciable change in the necrotising activity was observed when sera from patients were tested before and after corticosteroid treatment. The sera of 3 patients with typhoid fever in remission were tested for their capacity to induce a local Shwartzman phenomenon in the rabbit. All three sera were able to prepare the skin as well as provoke the reaction when injected intracutaneously in amounts of 0.2 to 0.5 ml followed 24 h later by an intravenous injection of 0.5 ml/kg. These sera were also able to prepare the skin for the provocative injection of *Proteus* endotoxin or to elicit the reaction in a skin site prepared with *Proteus* endotoxin. No such Shwartzman activity was noticed when serum was used which had been obtained from a patient with fresh typhoid fever. In accordance with LOVELL, PRYCE, and BOAKE[3] but in contrast to KLEMPERER et al.[6], no skin necrotising activity was observed when rheumatoid arthritis sera were injected intracutaneously in either rabbit or rat[9].

Zusammenfassung. Es wird eine semi-quantitative Abwandlung des Hautnekrosetests von BOAKE, PRYCE und LOVELL beschrieben. 108 Seren von Patienten, zur Hauptsache solchen mit primär-chronischer Polyarthritis, und 36 Seren von klinisch Gesunden wurden auf diese Weise am Meerschweinchen auf ihre Nekrose erzeugende Eigenschaft geprüft. Unter den pathologischen Seren erzeugten 89% eine positive Hautreaktion, wogegen weniger als 10% der Seren von Gesunden einen schwach positiven Ausfall der Hautreaktion hervorriefen.

R. JAQUES

Pharmazeutische Abteilung der CIBA Aktiengesellschaft, Basel (Switzerland), November 12, 1962.

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Ferrous Complexes in the Catalase Reaction

WIESNER[1] has recently suggested that new evidence from polarography supports the mechanism for catalase action put forward by WESTHEIMER[2]. According to WIESNER[3], HANUS[4] has shown that in the haemin-catalysed reduction of hydrogen peroxide[5,6] reversible haem-peroxide complexes ($Fe_h^{2+} - H_2O_2$) are involved. There are, however, some serious objections to WESTHEIMER's theory, and there is also a need for considerable caution in adopting the kind of analogy WIESNER proposes.

The polarographic evidence refers only to free haematin in strongly alkaline solution. Catalase itself[7] and blood haemolysates[5] remove the hydrogen peroxide 'wave' in the electrolytic reduction of oxygen by destroying the peroxide catalatically in solution (Equation 1):

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad (1)$$

But no intermediates active at the electrode in such systems have been detected. Even in the case of haemin, the only intermediates chemically identifiable are the ferrous (Fe_h^{2+}) and ferric (Fe_h^{3+}) forms. Under certain conditions[1], the half-wave potential of the catalysed reaction is that of the Fe_h^{2+}/Fe_h^{3+} couple. The oxidation of ferrous haem by peroxide may therefore play an important role in the catalysis, despite the calculated discrepancies in rate constants[1], because the apparent velocity constants obtained polarographically often exceed the true constants obtained by conventional chemical techniques[8]. Furthermore, under the conditions employed the rate limiting step may not be that of Equation 2, the usual reaction of haem with peroxide; other reactions can be involved in the reoxidation of the ferrous haem.

$$Fe_h^{2+} + H_2O_2 \rightarrow Fe_h^{3+} + OH^- + OH\cdot \qquad (2)$$

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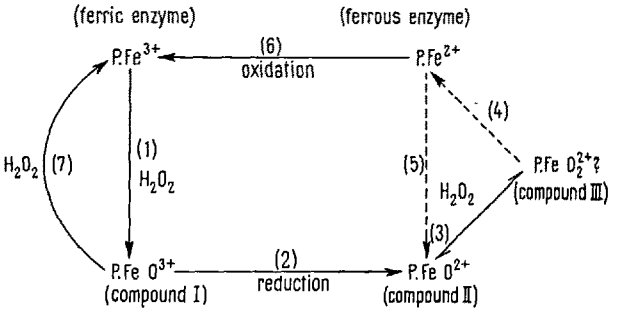
The known derivatives of Fe_h^{3+} and Fe_h^{2+} with peroxides are not readily reversible[9] and usually engage only in processes tending further to degrade the porphyrin ring[10]. Very strong reducing conditions are required to re-form the original haematin[11]. And the phenomena that induced LEMBERG[10] to propose the fleeting existence of a $Fe_h^{2+}-H_2O_2$ complex can probably be interpreted in terms of free radical reactions[12]. Thus although it is conceivable that short-lived derivatives of the kind postulated by HANUS[4] play a role at the surface of the electrode, they cannot be postulated as catalytic entities for the reaction in bulk solution.

The derivatives of catalase which occur in its reactions with peroxides[13] are probably ferric[14]. Although apparently not polarographically active[1,7], they are—unlike the derivatives of protohaematin[9] and protohaemochromogens[6]—readily reconverted to the (ferric) enzyme. WESTHEIMER's theory[3] is based upon the idea[12] that compound III of catalase is the oxyferrous state. This is now known to be improbable; thus ferrocatalase[15] and ferroperoxidase[16] autoxidise directly to the ferric state, while compound III decomposes to compound II[17,18].



—→ indicates observed reactions
- - → reactions postulated by WIESNER[1] and WESTHEIMER[2]

Theories of catalase action

If the reaction schemes of the usual theory of catalase action[12,14] and that of WESTHEIMER[2] are represented as in the Figure, then the oxygen-evolving steps postulated by the two theories are given by reactions (7) and (4) respectively. But the simple form of WESTHEIMER's theory must be rejected, for the following reasons:

(a) The catalatic reaction is unaffected by carbon monoxide and no other evidence for ferrous intermediates has been produced[12,14].

(b) No lag phase precedes O_2 evolution[19], as would occur if reactions (2) and (3) preceded the O_2-evolving step (compound II formation is slow[20]).

(c) The formation of compounds II and III inhibits the reaction and does not promote it. Reaction (3) is an equilibrium[17] and reaction (4) cannot be detected; thus compound III does not react with carbon monoxide[18].

(d) The initial reaction steady state shows the presence of ferric enzyme and compound I only (in accord with the participation of reactions (1) and (7))[12,14,20].

(e) Ferrous catalase is oxidized to ferric catalase by reaction (6) and reaction (5) cannot be detected[15]. Similarly, ferrylhaemoglobin (Hb.FeO^{2+}) can be formed directly from ferric but not ferrous haemoglobin[21]. For ionic iron, CAHILL and TAUBE[22] have proposed a mechanism similar to WESTHEIMER's schema (in which Fe^{IV} and Fe^{VI} states replace to a certain extent the $OH^{\cdot}$ and $HO_2^{\cdot}$ radicals of the usual formulation[23]). But even in this case alternative mechanisms which attribute a more central role to ferric iron are possible[24].

(f) Only H_2O_2 gives rise to compound III via reaction (3); compound I can however react with many other hydrogen donors[14], and catalase will oxidize these donors under its physiological conditions of activity[25].

If one tries to modify the theory of active ferrous complexes to fit the facts, one finds that: the reduction process (reaction 2) must be very fast; and the oxidation process (reaction 6) must also be fast. Hence reaction (1) will be more important than reaction (5). Furthermore, the compounds II (P.FeO^{2+}) and III (P.FeO_2^{2+}) postulated here cannot be the same as those observed directly. The 'catalytically active' compound II (if it exists) must be the derivative with the spectrum normally attributed to compound I; and it must retain the oxidizing equivalent used up in reaction (2), or the reaction would cease as soon as the sink of reducing equivalents was exhausted.

The modified theory is formally equivalent to the usual theory of reactions (1) and (7), except that it postulates a ferrous or ferryl state for the iron in 'compound I'. Such a view has been advocated by WINFIELD[26], but involves the difficulty that an unpaired electron of a free radical type would then be expected (the structure $Fe^{3+}HO_2^{\cdot}$). No such free radical has been detected[27]. The paramagnetism of compound I is more consistent with either a 'pentavalent oxidation state' of the iron[18] or a 'mixed spin state' involving ferric iron[28]. The action of co-ordinating ligands such as fluoride and acetate on compound I suggests that it retains the ferric structure of the free enzyme[29]. Similarly, the rate-limiting intermediate at high peroxide concentrations[30] is probably not an oxyferrous compound but another ferric compound, for instance a simple complex of hydrogen peroxide with compound I.

In summary: there is no known analogue of compound I in the reactions of free haematin, and hence those reactions cannot provide a model for catalase activity; there is no good evidence for reversible complexes of ferrous haematins with peroxide, sufficiently stable to act as catalytic

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intermediates; and deoxygenation reactions (reaction (4), Figure) cannot be a good model for catalase activity (as opposed to the catalatic activity of free iron salts[24]) because catalase compound I can react with numerous other hydrogen donors[14,25] whose oxidation products do not form complexes with ferrous iron.

Résumé. L'auteur critique l'idée que la polarographie démontra l'existence de complexes d'hématine ferreuse avec le peroxide, actifs dans la catalyse. D'autres expériences montrent que les dérivés de l'hématine et du peroxyde sont instables et provoquent la dégradation de la porphyrine. Les complexes de la catalase et du peroxyde ne sont pas actifs polarographiquement et contiennent probablement le fer à l'état ferrique. La théorie de WESTHEIMER sur l'action de la catalase ne s'accorde pas avec les faits démontrant que les dérivés ferreux n'apparaîssent pas dans la réaction et que les complexes avec le peroxyde, autre que le premier composé, sont inactifs. On ne peut pas établir d'analogie bien fondée entre la catalase proprement dite et l'hématine libre.

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An Enzyme(s) from a *Streptomyces sp.* to Prepare Mould 'Protoplasts'

During recent years it has been shown that enzymes from *Streptomyces sp.* are a useful tool for the study of bacterial structures. WELSCH[1] has recently reviewed the field and described the different organisms and the techniques being used by the different workers.

Several instances of 'protoplast' formation from hyphae of a number of mould species were recently reported as resulting from a destruction of mould cell-wall, in particular in the case of gut juice of the snail *Helixpomatia* inducing protoplast formation[2,3]. Reports from our laboratory show the formation of protoplasts like-structures from a large number of moulds prepared by the use of the gut juice of the snail named before[4] as well as the obtention of protoplasts from various yeast species employing an enzyme preparation (strepzyme) from *Streptomyces GM*[5]. Our aims are to test their potential activities upon the various mould components that are being isolated in this and other laboratories, since the results of such work might well enlighten our knowledge of fungal structures.

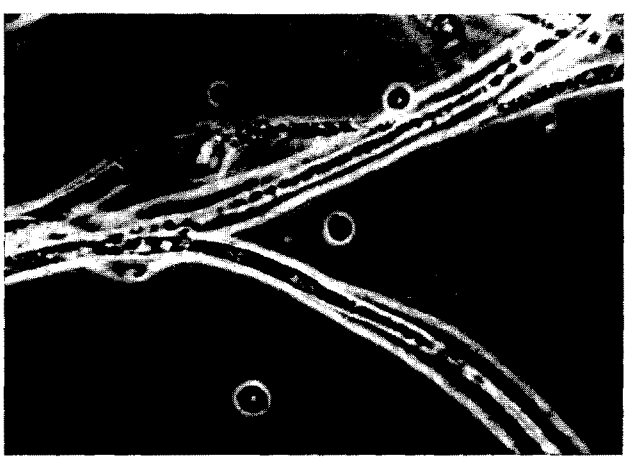
At the moment research is in progress devoted to a comparative study of the action of snail and Streptomyces enzymes upon several yeasts and moulds with the aim of finding out whether transformation into 'protoplasts' by these preparations occurs through similar mechanisms and whether differences are to be found in the response of various species and strains. In the following, we shall only show very briefly that the strepzyme, that is a suitable culture filtrate from our *Streptomyces sp.* strain GM, contains (one or various) principles acting specifically upon given species of fungi. The properties of this agent clearly show that it is a protein and an enzyme[6,7]. Studies are now in progress on identification of the enzyme activities to be found in the culture filtrate preparation of *Streptomyces GM* and will be reported elsewhere.

The study was carried out with the fungi *Mucor sphaerosporum, Penicillium italicum, Aspergillus nidulans, Fusarium culmorum, Verticillium hemileiae, Helmintosporium gramineum* and *Alternaria citri* which were submitted to the action of the *Streptomyces* enzyme. Transformation into 'protoplasts' under the influence of a suitable concentration of strepzyme was obtained with nearly all of them, *Alternaria citri* and *Helmintosporium gramineum* being the only exceptions. It is necessary to state that the rate, completeness and extent of the transformation, vary widely according to the organism under consideration.

Young hyphae of each of the above-named mould species were obtained by inoculating a small volume of a spore suspension of slant cultures into liquid Czapek medium. After a growth period of 18–24 h at 28°C, under vigorous aeration, the mycelium was harvested by centrifugation and thoroughly washed with distilled water. Then the mycelium was resuspended at a low density in 0.1 *M* phosphate buffer pH 6.8 containing 0.8 *M* mannitol. It should be noted that, in our experiments, no Mg^{++} or Ca^{++} were added to the medium.

For conversion of the mould hyphae into 'protoplasts', 0.2 ml of the enzyme preparation was added per ml of incubation mixture. After 3 h incubation at 30° with gentle shaking, the protoplasts began to emerge from the more susceptible mould species, v.g. *F. culmorum.*

When mycelial suspensions, submitted to the action of a suitable concentration of strepzyme in the presence of



The Figure shows 'protoplasts' of *Fusarium culmorum* of various sizes and some empty cell walls after $4^1/_2$ h of incubation under the conditions described in the text.

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