filter paper and completely volatile solvents have been used. A slit-width of 0.25 in and a scanning rate of 12 in/h were used throughout. Under these conditions the printer prints out counts/1.25 min/spot. The system as described includes background only after the gate has opened and does not include the radioactivity in that part of the peak below the threshold. These two factors require corrections of opposite sign and there is a tendency for them to cancel each other, with a net loss of about 100 counts/min per peak when the threshold is set at twice the background count. In addition to this correction, there is a percentage loss that results from the geometry, the window, and the absorption losses caused by the filter paper. These 3 factors combined to give an efficiency that varied from 37 to 45 % when the printed counts were compared with counts/min from a windowless gas-flow counter calculated for samples at infinite thinness over a range of 500 to 20,000 counts/min. These data were independent of the time of development in solvent over a range of 3 to 9 h. It is clear that the significance of the 100 counts/min correction increases when peaks contain less than 1000 counts/min and decreases at higher levels and that maximum accuracy can be obtained by using a calibration curve which empirically combines all factors for any given system.

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The isolation of iminazole acetaldehyde as the only metabolite of the action *in vitro* of placental histaminase on histamine

In a recent paper[1] a chemical synthesis of the 2,4-dinitrophenylhydrazone of iminazole acetaldehyde was reported as well as the identification of iminazole acetaldehyde as the only metabolite of the action *in vitro* of histaminase from hog kidney on histamine. Since the literature concerning the enzymic destruction of histamine by oxidative deamination has recently become very involved[2–9], it appeared to be important to investigate whether human placental histaminase would yield during its action on histamine *in vitro* the same metabolite as hog kidney.

For the preparation of a placental-histaminase extract a normal placenta, soon after birth, was mechanically minced to a fine brei, and to the weighed wet pulp was

added with mechanical stirring the double amount of 1 % NaCl. After straining through muslin the pulp was extracted for a second time with the same amount of 1 % NaCl as before. The combined filtrates were centrifuged, and to the clear, measured supernatant 42.4 g $(NH_4)_2SO_4$/100 ml (0.6 satn.) was added with mechanical stirring. To the precipitate formed, filtered over fluted filters, 200 ml 0.02 *M* phosphate buffer, pH 6.8, were added. The slightly turbid solution was dialysed against tap water for 2.5 h. This highly active enzymic extract was used for the study of the action of placental histaminase on histamine. By means of various techniques, applied in investigations of histaminase from hog kidneys[1], it was found in the present work that by the action on histamine of placental histaminase like of that of hog kidney histaminase only one metabolite, an iminazole derivative, is formed. In paper-chromatographic assays the metabolite gave a single diazo-stained spot, which clearly showed characteristics suggestive of iminazole acetaldehyde. When subjected to oxidation, either enzymic with pure xanthine oxidase (60 min, 37°) or non-enzymic with 0.1 *N* $KMnO_4$ (5 min, 50°), the product yielded iminazole acetic acid. As in previous experiments, an attempt was made to identify this reaction product of the action of placental histaminase on histamine by the isolation of one of its derivatives.

Reaction mixtures in 100-ml Erlenmeyer flasks each contained 10 ml of the dialysed placental extract, described above, 4 ml phosphate buffer (pH 6.8), 0.8 ml of a diluted solution of catalase (L. Light, Ltd.), corresponding to about 0.6 mg catalase, and 4.4 ml of a histamine solution containing 44 mg histamine dihydrochloride. Three such reaction mixtures containing in total 132 mg histamine dihydrochloride or 79.6 mg histamine base, were separately incubated at 37° for 75 min. The combined incubation mixtures were brought into reaction with 2,4-dinitrophenylhydrazine according to the method previously described. The orange-red crystals which formed were filtered and washed with 50 % alcohol (m.p. of dried crystals was 112°). On recrystallisation from 50 % acetone, 189.2 mg of golden silky needles (m.p. 124–126°) were obtained, very similar to iminazole acetaldehyde 2,4-dinitrophenylhydrazone, prepared in previous work[1] by two different routes, a synthetic and an enzymic one. Since the m.p. of the new preparation did not change on further recrystallisation from 50 % alcohol or 50 % acetone and remained undepressed by the admixture of preparations of iminazole acetaldehyde 2,4-dinitrophenylhydrazone of either synthetic or enzymic origin, or by the simultaneous admixtures of both, the further purification on bentonite-kieselguhr (4:1) columns, used previously, appeared to be superfluous. For a further identification of the compound a paper chromatogram was prepared in parallel with that of the authentic preparation of synthetic iminazole acetaldehyde 2,4-dinitrophenylhydrazone and also with one of enzymic origin. Fig. 1 shows the completely identical paper-chromatographic behaviour of the three compounds and of a mixture of these substances. In all instances yellow spots were obtained, R_F 0.86 (propanol–ammonia, Whatman No. 4 paper, developing time 5 h). Identical results with all these preparations were also obtained in another solvent system (R_F 0.62(10 % Glycerol v/v, 10 % acetic acid v/v, 10 % phenol w/v, Whatman No. 1 paper). Finally, since 189.2 mg of the pure 2,4-dinitrophenylhydrazone of iminazole acetaldehyde have been isolated from 79.6 mg histamine (91 % of the theoretical yield) this seems to indicate that the placental histaminase has changed histamine with the stoichiometric formation of a single metabolite, iminazole acetaldehyde.

Recently, LINDAHL, LINDELL, NILSSON, SCHAYER AND WESTLING[10] studied the metabolism of [^{14}C]histamine in plasma during human gestation. On the basis of results obtained by radioisotope methods, applied to plasma of women during their last weeks of pregnancy, these workers suggest that human pregnancy plasma inactivates histamine enzymically by oxidative deamination, and that methylation

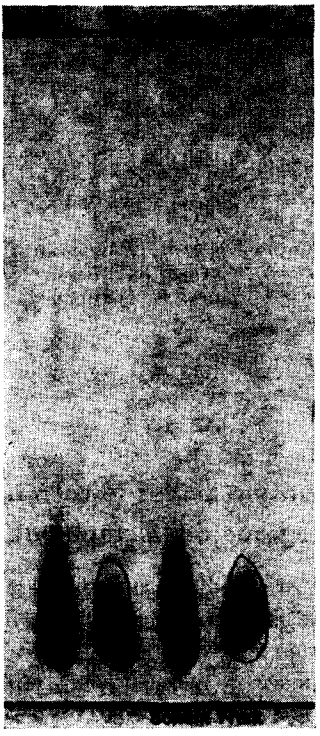

Fig. 1. Paper chromatogram of the iminazole acetaldehyde 2,4-dinitrophenylhydrazone. S, synthetic compound; P, compound obtained by enzymic action of placental extracts; K, compound obtained by enzymic action of hog-kidney extract; Mix., mixture of S, P, and K.

of histamine which SCHAYER AND COOPER[11] supposed to be the principal route of histamine metabolism in man is apparently not the route of histamine degradation by pregnancy plasma.

The evidence reported in the present paper of the stoicheiometric formation of a single metabolite, identified as iminazole acetaldehyde, in the attack *in vitro* of histaminase from placenta (the site of histaminase formation in human pregnancy[12,13]) on histamine seems to indicate that in human pregnancy histamine is being destroyed mainly by oxidative deamination.

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Signification des amino acides N-terminaux pour l'action biologique de la staphylokinase

Dans une note antérieure on a relaté les résultats de l'analyse chimique d'un préparé de staphylokinase purifiée, de même que les résultats des déterminations des amino acides N-terminaux et C-terminaux[1].

On sait que la staphylokinase, de même que la streptokinase, sont des activateurs du plasminogène, protéine plasmatique, inactive et qui, sous l'action de ces activateurs, est convertie en plasmine, enzyme à action fibrinolytique.

L'action activante a été interprétée, soit comme le résultat d'une combinaison stoechiométrique directe entre l'activateur et le plasminogène[2], soit comme une réaction enzymatique en deux étapes[3], la première étape comportant une réaction entre l'activateur d'origine bactérienne (staphylokinase, streptokinase) et un cofacteur plasmatique. Cette réaction réalise la formation d'un autre activateur, qui, dans une deuxième étape, transforme par voie enzymatique le plasminogène en plasmine.

Le mécanisme d'activation par la staphylokinase comporte en tout cas une réaction intermédiaire entre ce facteur enzymatique et son substratum.

Tenant compte de la structure protéique de la staphylokinase et du fait que les amino acides terminaux, par leur position spéciale dans la chaîne polypeptidique, pourraient intervenir de manière directe dans le comportement chimique et biologique de ce facteur enzymatique, nous avons entrepris l'étude de l'influence du blocage des aminoacides N-terminaux sur l'action activante spécifique exercée par la staphylokinase sur le plasminogène. La présente note expose les résultats de cette étude.

Les amino acides N-terminaux, identifiés dans la staphylokinase purifiée, ont

Abbréviation: DNFB, dinitrofluorobenzène; DNP, dinitrophényl.