and $E_2$, (Figure 2). The appearance of sharper and narrower esterase zones seems to be due to the more efficient sieving by much smaller gel pores in $7^1/_2$% gel compared with the gel pores of 5% gel. In addition to sharper resolution, the carboxylesterase $E_{10}$ (which had remained near the origin in 5% gel formulation) moved freely through the courser 3% gel portion and was concentrated at the boundary of the $7^1/_2$% gel portion. In addition to the improved resolution, quantitative differences were apparent on the gel between esterases of the normal and resistant strain. Supernatants of susceptible and resistant strains, and of the same concentration were separated on the same gel. The quantitative differences observed were found repetitive and thus in my opinion fairly reliable. Of the 10 carboxylesterase zones in the normal strain, 8 esterases were found in the SKA strain. The 2 esterase ($E_6$ and $E_7$) which could not be resolved visually, perhaps occur in greatly reduced quantity and thus did not resolve from the closely occuring and highly active esterase $E_8$. This is further suggested by a rather broader esterase zone $E_8$ in the densitometric tracing of the resistant strain (Figure 2). Of the observed 8 esterases in the resistant strain all, with the exception of $E_2$ (second from the anode) showed a reduced level of esterase activity, as can be seen from the densitometric tracings. This is in agreement with the fact that the resistant strain showed one quarter hydrolysis of ethylbutyrate compared with the susceptible strain[5,6]. The 'low esterase' phenomenon therefore appeared in the SKA strain, not due to the absence of a single and most active anodic band as reported earlier but due to the absence of 2 esterases and reduction in the activity level of all the other esterases with the exception of one[2,3,10].

Esterase $E_2$ appeared to be unusually more active in the resistant strain. This was unexplainable when taken into account that only about a quarter of the esterase activity is present in the SKA strain and that all the other bands show considerably reduced activity. Mobility measurements of phosphate hydrolyzing bands in the 2 strains showed that the anodic phosphatase band is the same as aliesterase $E_2$; and showed higher phosphatase activity in the resistant strain than in the susceptible. Similarly the phosphatase band near the cathode appeared to be identical with carboxylesterase $E_{10}$. It was therefore concluded that the 2 phosphate hydrolyzing enzymes are identical with esterases $E_2$ and $E_{10}$ respectively. This is supported by the following additional facts: 1. It has been reported that phosphatase type enzymes of the housefly show some esterasic activity towards aliphatic esters and also tend to be inhibited by organophosphates[11]. 2. These enzymes were inhibited by $Mg^{++}$ ions and similar results have been reported for carboxylesterases of SKA strain[12].

From these studies, therefore, it appears that increased hydrolysis of naphthyl phosphate in the SKA strain is not due to an additional phosphatase enzyme, but is due to an increased production of one of the esterases already present in the susceptible housefly. Clearly more work is required to elucidate the precise involvement of this enzyme.

*Zusammenfassung.* Die Carboxyesterase-Isozyme von 2 *Musca domestica*-Stämmen wurden analysiert: Die Esteraseaktivität im organophosphat-resistenten Stamm ist bedeutend geringer als im suszeptiblen Stamm. Hingegen ist eines der 10 Isozyme mit Phosphataseaktivität im resistenten Stamm aktiver oder in höherer Konzentration vorhanden als im suszeptiblen Stamm, was die Annahme einer direkten Resistenz-Beziehung nahelegt.

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[14] Special thanks to Dr. C. T. LEWIS for his help, support and encouragement throughout this study.

## Human Placental Alkaline Phosphatase, an Inhibitor of Hemagglutination by PR8-Influenza A Virus

Human placental alkaline phosphatase (EC.3.1.3.1) is a sialoglycoenzyme[1-3] containing terminal sialic acid moities. The enzyme preparation, especially its heavy molecular weight variants have been reported[4,5] to inhibit hemagglutination by Toolan's $H_1$ virus isolated from a human neoplasm ($HEp_I$). The present work was undertaken to determine if purified placental alkaline phosphatase preparations containing heavy molecular weight B variants[6] can inhibit hemagglutination by the well-characterized PR8-influenza A virus and also to ascertain the effects of altering alkaline phosphatase by treatment with proteolytic enzymes, neuraminidase, oxidizing agents, for example sodium metaperiodate and precipitation of the enzyme with specific antiserum on its hemagglutination inhibition (HA-I) titer.

*Materials and methods.* PR8-influenza A virus was a standard preparation supplied by the Viral and Rickettsial Registry and Distribution Center of the American Type Culture Collection, Rockville, Md. 1 hemagglutinating unit is that amount of virus which causes agglutination of 0.2 ml of 0.4% (v/v) adult chicken erythrocytes (Microbiological Associates, Bethesda, Md.). Hemagglutination (HA) and hemagglutination inhibition (HA-I) titrations were carried out as reported in previous communications[4,5]. The method for purifying alkaline phosphatase was similar to that of GHOSH and FISHMAN[6]. Alkaline phosphatase activity of enzyme preparations was assayed as described in an earlier publication[6]. Specific activity of alkaline phosphatase is expressed in μmoles of

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Table I. Enzyme activities and HA-I titers of placental alkaline phosphatase preparations during purification

| Steps in purification | Specific alkaline phosphatase activity (μmoles phenol/min/mg protein) [a] | Specific hemagglutination inhibition titer of alkaline phosphatase (HA-I titer/mg protein × $10^{-3}$) [b] |
|---|---|---|
| Supernatant of placental homogenate | 0.3 | 4 |
| *n*-Butanol extract of homogenate | 9.3 | 38 |
| 30–60% Ammonium sulfate fraction | 23.0 | 67 |
| Sephadex G-200 eluate | 164.0 | 136 |

[a] Enzyme preparations were derived from a single placenta but purification was carried out in several batches. [b] HA-I titers were determined with 8 hemagglutinating units of PR8 influenza A virus in 25 m*M* *Tris*-HCl buffer, pH 8.4 containing 150 m*M* saline.

phenol released per min per mg protein. The amounts of protein in different alkaline phosphatase fractions were measured by the method of LOWRY et al.[7]. Neuraminidase treatment was according to the method of GHOSH, KOTOWITZ and FISHMAN[2]. Sialic acid was measured by the method of WARREN[8]. Periodate treatment of the enzyme for 16 h at pH 4.5 was done by a standard procedure[9,10]. Placental alkaline phosphatase preparations were exposed to solutions of crystalline chymotrypsin, trypsin and papain at pH 7.1 for 18 h at 37 °C. Antibody against the placental enzyme was prepared by the method of GHOSH and COX[11].

*Results and discussions.* Table I shows specific alkaline phosphatase activities and HAI titers during purification of an alkaline phosphatase preparation derived from a single human placenta. The results indicate that as the purity of alkaline phosphatase increases there is a rise in the specific HA-I activity. The most active HA-I fractions from Sephadex G-200 gels with the heavy molecular weight isoenzymes had a specific enzyme activity of 164 units and a HA-I titer of 136,000 per mg protein. Figure 1 shows a chromatogram obtained during purification of human placental alkaline phosphatase on Sephadex G-200 gel. The peaks of enzyme activities of early Sephadex G-200 eluates contain the heavy molecular weight (B) variants[6] and are fairly superimposable on those of HA-I titers and sialic acid content. The earliest fractions, 28 to 32 in Figure 1 have the heavy molecular weight, slow moving isozymes B[6] and demonstrate high HA-I activities. The later fractions (33 to 50) contain low molecular weight variants A[6] and exhibit consideraably less or no HA-I activity. Typical hemagglutination patterns of PR8-influenza A virus before and after incubation with human placental alkaline phosphatase preparations are presented in Figure 2.

The release of sialic acid residues from the enzyme glycoprotein by incubation with *Vibrio cholerae* neuraminidase led to a reduction of HA-I titer (Table II). The HA-I titer decreased with increasing neuraminidase concentration. The oxidation of the *cis*-hydroxyl groups of the carbohydrate moities in the glycoprotein by treatment with sodium meta-periodate also caused a substantial decrease in the HA-I titer of the enzyme (Table II). Treatment of alkaline phosphatase preparations with 1 of

Table II. Effects of neuraminidase, sodium periodate, chymotrypsin, trypsin and papain on HA-I titer of placental alkaline phosphatase [a]

| Reagent | HA-I Titers of alkaline phosphatase preparations (%) |
|---|---|
| None | 100 |
| Neuraminidase (0.05 U) | 50 |
| Neuraminidase (0.5 U) | 12 |
| Neuraminidase (5 U) | 3 |
| Sodium periodate (7.5 m*M*) | 12 |
| Sodium periodate (75 m*M*) | 3 |
| Chymotrypsin (0.125 mg/ml) | 25 |
| Chymotrypsin (2.0 mg/ml) | 0 |
| Trypsin (0.5 mg/ml) | 25 |
| Trypsin (2.0 mg/ml) | 0 |
| Papain (0.125 mg/ml) | 24 |
| Papain (2.0 mg/ml) | 0 |

[a] Specific activity 140 units per mg protein.

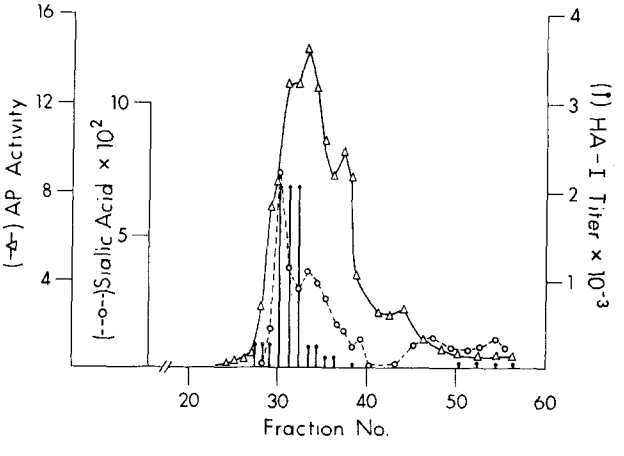


Fig. 1. Enzyme activity, hemagglutination inhibition titers and sialic acid content of human placental alkaline phosphatase fractions obtained by Sephadex G-200 gel filtration. Alkaline phosphatase (AP) activity (△) is expressed in μmoles phenol per ml per min. Sialic acid (0) is given in μmoles per ml as determined after hydrolysis with 100 mN sulfuric acid at 80 °C for 1 h. HA-I titer/0.1 ml (●—) was measured using 4 HA units of virus and is expressed as the reciprocal of the highest dilution of the enzyme completely inhibiting agglutination of adult chicken erythrocytes. The 60% ammonium sulfate fraction used for gel filtration was prepared by the precipitation method as previously described[6].

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3 proteolytic enzymes, chymotrypsin, trypsin or papain led to significant lowering of the HA-I titer (Table II). Human placental alkaline phosphatase preparations were found to inhibit agglutination by PR8-influenza A virus of human erythrocytes as effectively as agglutination of chicken red blood cells. The antigen-antibody precipitate formed by reacting purified placental alkaline phosphatase with specific antiserum exhibited alkaline phosphatase activity as well as HA-I titer of 184 units per unit of enzyme activity.

Certain malignant tumors and HeLa cells in tissue culture contain an alkaline phosphatase with physical, chemical and immunologic properties that closely resemble the placental forms of the enzymes[12,13]. $HeLa_{65}$ cell alkaline phosphatase (purified 220-fold) exhibited viral hemagglutination inhibitory potency. The present investigation[14] indicates that the high molecular weight variants of placental alkaline phosphatase are potent inhibitors of influenza virus hemagglutination. The inhibitory capacity of the enzyme seems to be dependent both upon sialic acid residues and molecular size.

*Résumé.* La phosphatase alkaline du placenta humain est un sialyglycoenzyme qui inhibe l'hémagglutination (HA) produite par le *Virus influenzae* A (PR-8). L'enzyme de poids moléculaire élevé donne un titre HA-I beaucoup plus grand que les enzymes de poids moléculaire inférieur L'élimination de l'acide sialique de l'enzyme par traitement à la neuraminidase ou par oxydation des carbohydrates avec du périodate ou par protéolyse amènent une réduction prononcée du titre de HA-I.

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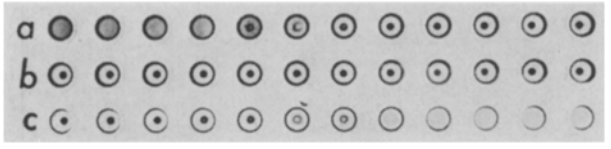


Fig. 2. Effect of placental alkaline phosphatase on hemmagglutination caused by PR8-influenza A virus. a) Serial dilutions of 32 units of virus plus red cells (hemagglutination is observed up to the 4th dilution or 4 units of virus) b) Red cells alone in buffered saline (control) c) 8 units of virus previously incubated with serial dilutions of placental alkaline phosphatase and red cells (hemagglutination-inhibition is observed through the 7th well or a dilution of 1:128 of the enzyme).

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## The Kinetics of Cerebral RNA Synthesis in Relation to the Route of Injection

Many kinetic studies exist on the metabolism of cerebral RNA in the living animal. These have generally utilized radioactive RNA precursors such as inorganic phosphate or nucleotides which have been injected subcutaneously[1-3] intracranially[4,5] or into the circulatory system[6-8]. After various time intervals animals are killed and the extent of isotopic incorporation into RNA is related to the amount of radioactivity remaining within the acid soluble pool. In the search for a relation of cerebral macromolecule synthesis to brain function, relatively minor differences in the rate of RNA synthesis in animals under differing experimental conditions have been reported[2,3,6].

The kinetics of the synthesis of labelled cerebral RNA from $^3$H-uridine administered by various routes in the young chick are reported. The data suggests that rates of cerebral RNA synthesis are not readily determinable, and that a variety of parameters must be taken into account.

*Methods and materials.* 1–4-day-old chicks were injected with 5-$^3$H-uridine (27.1 Ci/mmol) in 3 different ways: 1. by s.c. injection into the scruff of the neck of 50 $\mu$Ci $^3$H-uridine. 2. by intracardiac injection of 50 $\mu$Ci $^3$H-uridine. 3. by intracranial injection of 5 $\mu$Ci $^3$H-uridine into the right cerebral hemisphere. After various time intervals, chicks were decapitated and the right cerebral hemisphere rapidly dissected out. Hemispheres were then homogenized in 5 ml 0.32 *M* sucrose at 0 °C, and this homogenate was centrifuged at 1000 *g* for 10 min. 0.5 ml 50% (w/v) TCA was then added to the resulting cytoplasmic supernatant and the precipitate was resuspended in cold 5% TCA. Both supernatant and precipitate were then centrifuged at 0 °C and 1000 *g* for 10 min. Unincorporated radioactivity was assayed in samples of the supernatant from the TCA-treated cytoplasmic fraction. The precipitates were washed twice more with cold 5% TCA, once with ethanol, and were then incubated for 2 h at 37 °C in 2 ml 0.1 *N* NaOH. This suspension was then brought to a pH below 3.0 with perchloric acid and the precipitate of protein and DNA was removed by centrifugation. Radioactivity within the supernatants containing hydrolysed RNA was determined.

The proportion of radioactivity remaining in nucleotides in the acid-soluble TCA supernatant was assayed by absorption on to charcoal. 1 ml aliquots of these supernatants were taken and made up to 10 ml in 0.1 *N* HCl. Around 5 mg of charcoal (acid washed) were stirred into each sample which was then centrifuged. Under these conditions, uridine and its phosphorylated nucleotides bind to the charcoal[9,10]. 1 ml samples of the supernatants were counted in order to determine non-nucleoside,

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