labile NANA. A similar behaviour, however, was already described for gangliosides GM1, GD1a (GD1b?) and GT1b by SUZUKI and KOREY[6]. It may be provisionally explained by postulating the existence of a different pool of precursors for the resistant and the labile NANA.

The total incorporation of radioactivity into ganglioside GQ1, in 8-day-old rats, 8 h after injection, is 25,000 cpm/μM. Under the same conditions, which were reported to be optimal also for the other gangliosides, the specific radioactivities of gangliosides GM1, GD1a, GD1b and GT1b were, respectively: 13,500; 18,000; 8,600; 13,300 cpm/μM. This means that none of the above-mentioned gangliosides, which are chemically simpler, can be supposed to act as precursor of GQ1. In consequence, a specific and separate enzyme system is required for the biosynthesis of GQ1. This assumption is in agreement with the current concept[7] on the biosynthesis of gangliosides, postulating a specific biosynthetic system for each ganglioside, completely apart from the cellular structures (the neuronal membranes) into which gangliosides accumulate. According to this concept the higher specifc activity of GQ1 observed (possibly the simple expression of a higher turnover of GQ1) may be due to a higher ratio between the amount of GQ1 produced (in labelled form) by its biosynthetic system and the amount of GQ1 present in the neuronal membranes. In other words the 'dilution' of the radioactive GQ1 in the total pool of GQ1 is lower than in the case of the other gangliosides.

Riassunto. L'incorporazione di glucosio-U-C^{14}, introdotto per via i.p., nel tetrasialoganglioside GQ1 del cervello di ratto è massima all'8° giorno di vita e dopo 8–10 ore dall'iniezione. La radioattività specifica del GQ1 è notevolmente superiore a quella riscontrata nei gangliosidi GM1, GD1a, GD1b e GT1b, il che esclude che questi siano precursori del GQ1. L'acido sialico neuraminidasi stabile è più radioattivo di quello neuraminidasi labile: ciò indica un diverso impegno metabolico dei due tipi di acido sialico.

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An Electrophoretic Study of the Negative Correlation of Certain Carboxylesterases to Insecticide Resistance in *Musca domestica* L.

Carboxylesterase (E.C.3.1.1.1.), a major hydrolase has been shown to be negatively correlated with organophosphate resistance in the house-fly[1]. Several attempts have since been made to characterize it from other hydrolases and to demonstrate such differences electrophoretically[2–4]. Although the patterns from these studies indicated variation between and within strains, no clearcut differences were demonstrated between standard susceptible and low-esterase resistant strains.

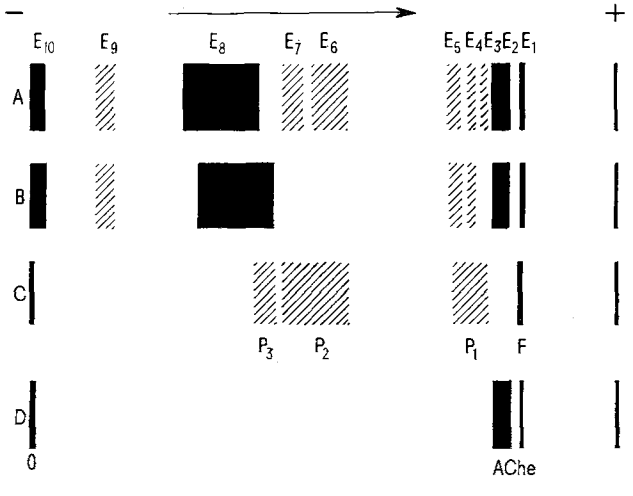

Fig. 1. Comparative zymogram of carboxylesterases (E_{1+2}-E_{10}) acetylcholinesterase (AChE) and peptidases (P_1-P_3) in a susceptible and an organophosphate resistant strain of housefly, separated in 5% gel. A) Carboxylesterases of susceptible strain, SRS. B) Carboxylesterases of resistant strain, SKA. C) and D) Peptidases and acetylcholinesterase of the susceptible and resistant strain. O, sample slot and F) indicates the marker dye front (10 cm). The arrow indicates the direction of protein migration.

An interesting strain of housefly, SKA, has been selected for several years with diazinon pressure at Rothamsted Experiment Station (Harpenden, England) and has developed resistance to a number of organophosphates and high cross-resistance to a number of chlorinated hydrocarbons[5]. My initial studies of the carboxylesterases of the SKA strain showed that the 'low esterase' activity in this strain is entirely due to carboxylesterase and not to other hydrolases such as lipase or peptidase[5]. Low carboxylesterase activity observed in the resistant strain was due to a proportionate decrease in head, thorax, abdomen and gut[6]. The present study was aimed at the further characterization and quantitation of various carboxylesterases of the SKA strain, separated electrophoretically on polyacrylamide gel. A WHO susceptible strain, SRS, obtained from University of Pavia, Italy, was used as a standard reference strain.

Materials and methods. One hundred 4-6-day-old decapitated houseflies were homogenized in cold (2–4 °C) in 4 ml of 0.1 M phosphate buffer adjusted to pH 7.0. The homogenate was centrifuged at 17,500 g for 20 min. The Table shows the results of centrifuging fly homogenates. The supernatant was used directly for electrophoresis of carboxylesterases, phosphatases and peptidases. For the separation of acetylcholinesterase, 100 fly-heads were homogenized in 4 ml of 0.1 M phosphate buffer and

[1] K. VAN ASPEREN and F. J. OPPENOORTH, Entomologia exp. appl. *2*, 48 (1959).
[2] H. H. W. VALTHUIS and K. VAN ASPEREN, Entomologia exp. appl. *6*, 79 (1963).
[3] D. B. MENZEL, R. CRAIG and W. M. HOSKINS, J. Insect Physiol. *9*, 479 (1963).
[4] W. J. COLLINS and A. J. FORGASH, J. Insect Physiol. *14*, 1515 (1968).
[5] S. AHMAD, Comp. Biochem. Physiol. *32*, 465 (1970).
[6] S. AHMAD, Comp. gen. Pharmac. *1*, 273 (1970).

Distribution[a] of carboxylesterase activity[b] in the supernatant and particulate fractions[c] of housefly homogenates

Enzyme Source	Housefly Strain	Hydrolysis of ethyl butyrate (μmole/30 min) Whole homogenate	Supernatant	Particulate
Head	SKA	0.75	0.1	NA[d]
Thorax	SKA	4.23	1.5	NA
Abdomen	SKA	4.4	2.57	NA
Decapitated fly	SKA	8.7	4.05	4.65
Decapitated fly	SRS	34.4	16.7	17.7

[a] Activity was recorded as the volume (μl) of 0.05 *M* NaOH required to neutralize butyric acid liberated by the action of carboxylesterase on ethylbutyrate. This was converted to μmole of ethylbutyrate hydrolyzed[5,6]. [b] The incubation was carried out at 37 °C. [c] See 'Material and methods' section for details on the procedure of homogenization and centrifugation to obtain particulate and supernatant fractions. [d] Activity not assayed.

centrifuged at 300 *g* for 20 min. Supernatant was used directly for electrophoresis.

Two types of polyacrylamide gel formulations were used. The first type was 5% (w/v); the second was a variable gel having an initial portion of 1.5 cm length of 3% (w/v) followed by a main running gel of $7^1/_2$%, an adaptation to slab electrophoresis of the system recommended for disc electrophoresis[7]. 100 μl of enzymatic extract (equivalent to 2.5 flyheads or headless flies) were used in each gel slot. The apparatus and the electrophoretic techniques, such as electrophoretic buffer, electrical conditions were essentially those used by PRICE[8].

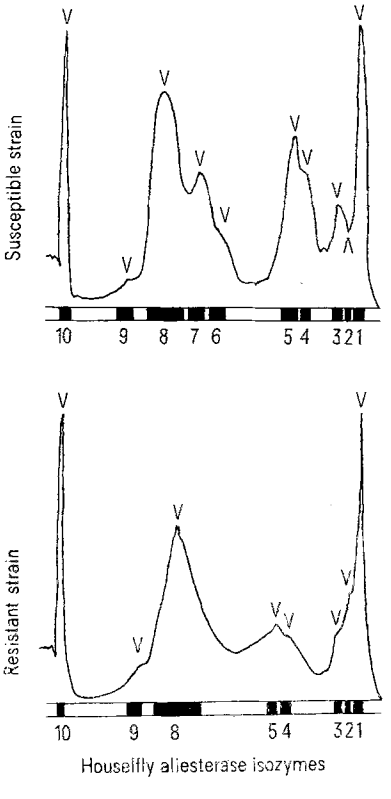

Fig. 2. Densitometric tracings and zymograms of aliesterases of the susceptible, SRS strain, and the organophosphate resistant, SKA strain, separated in variable gel (3 and 7.5%) gel. Numbers 1–10 designate carboxylesterases E 1–10 as in the text. E_{10} is at the interface of 3 and 7.5% gel; all the other esterases are within the 7.5% gel. The arrow indicates the direction of protein migration.

Carboxylesterase were visualized by staining for 20 min with 0.25% (w/v) solution of 1-naphthylacetate in 10% aqueous acetone. Afterwards the gel was incubated with 0.1% (w/v) diazo-dye, Fast Blue RR in 0.1 *M* phosphate buffer of pH 7.0. Upon development of deep brown colored esterase bands in 40 min, the gel was washed in 2 changes of distilled water and stored in 7% acetic acid.

Esterases were characterized from other enzymes by their inhibition by the organophosphate, tetraethylpyrophosphate (TEPP) at 1×10^{-5} *M*. Acetylcholinesterase (E.C.3.1.1.7.) was distinguished from carboxylesterase (E.C.3.1.1.1.) by its sensitivity to 1×10^{-5} *M* eserine sulfate. Arylesterase (E.C.3.1.1.2.) are not inhibited by either of these compounds at this concentration[9]. Peptidases were located by using 0.1% (w/v) DL-alanine-B-naphthylamide solution in place of 1-naphthylacetate. Phosphatase were stained with 0.4% sodium-1-naphthylphosphate and the diazo-dye, Garnet GBC salt, at pH 9.1. Marker dye, bromothymol blue was used to determine solvent front. Fresh gels were scanned by a double beam recording microdensitometer MK III as made by Joyce, Lobel & Co., Ltd., U.K.

Results and interpretations. The results are based on repeatedly consistent (3 times) separations on the mobility of each zone.

A) The results obtained in 5% gel slabs were as follows: Carboxylesterase bands were distinguishable from other enzyme bands due to other closely related hydrolases (Figure 1). A total number of 9 zones corresponding to 10 esterases were detected in the susceptible strain (Figure 1). Carboxylesterase E_1 appeared as the densest esterase band near the marker dye front. In this gel system E_2 cannot be distinguished from E_1, though the two were discriminated in the variable gel system as described later. In comparison, it will be seen from Figure 1 that in the resistant strain the esterases E_6 and E_7 were absent. Visual comparisons of the zone density indicated a reduction in the activity of every band.

B) The results of electrophoretic separation using variable gel formulation are as follows: Improved resolution was obtained of all the esterase bands, but in particular the first zone was clearly resolved into the 2 esterases E_1,

[7] L. ORNSTEIN, Ann. N.Y. Acad. Sci. *121*, 321 (1964).
[8] G. M. PRICE, Lab. Pract. *17*, 467 (1968).
[9] K. B. AUGUSTINSSON, Ann. N.Y. Acad. Sci. *94*, 844 (1961).

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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1 October 1973.

[10] K. VAN ASPEREN and M. E. VAN MAJIZK, Nature, Lond. *205*, 1291 (1965).
[11] K. VAN ASPEREN, Biochem. Pharmac. *3*, 136 (1960).
[12] H. H. SHATOURY and T. J. WOODAGE, Chemy. Ind. *1968*, 1315.
[13] Supported by Colombo Plan Fellowship financed by the British Council. This work was undertaken at the Field Station of Imperial College of Science and Technology, London, England.
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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

[1] N. K. GHOSH, S. S. GOLDMAN and W. H. FISHMAN, Enzymologia *33*, 113 (1967).
[2] N. K. GHOSH, L. KOTOWITZ and W. H. FISHMAN, Biochim. biophys. Acta *167*, 201 (1968).
[3] N. K. GHOSH, Ann. N. Y. Acad. Sci. *166*, 604 (1969).
[4] N. K. GHOSH and M. USATEGUI-GOMEZ, Biochim. biophys. Acta *177*, 565 (1969).
[5] M. USATEGUI-GOMEZ, N. K. GHOSH and H. W. TOOLAN, Proc. Soc. Soc. exp. Biol. Med. *130*, 1128 (1969).
[6] N. K. GHOSH and W. H. FISHMAN, Biochem. J. *108*, 779 (1968).