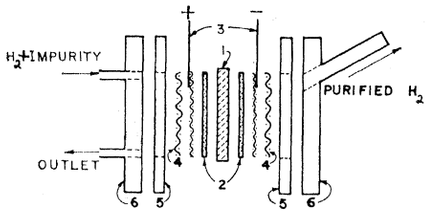

Fig. 1. Exploded section of model hydrogen purification cell: 1, saturated filter paper or membrane; 2, electrodes; 3, potential delivery screens; 4, spacers; 5, gaskets; 6, plastic casing.

Some typical performance data are given in Table 1. The hydrogen purification cell performs quite satisfactorily over a variety of conditions. Two amperes per minute is equivalent to about 15 cm^3 of hydrogen gas per minute. If speed of purification is important, electrode area and membrane resistance as well as temperature are readily altered. The calculated rate of purification was equal to the observed rate on the basis of coulombs passed through the cell. The results with Manufacturer's gas, containing carbon monoxide, indicate some poisoning of the hydrogen electrode. It is desirable to minimize the concentration of such gases in the impure gas stream or to use poison-resistant electrode catalysts.

Results with nitrogen-hydrogen mixtures are of interest since they suggest the catalytic decomposition of ammonia as a source of hydrogen. Since several hydrogen purification cells can be connected in series or in parallel, the purification process itself can be self-powered by using some hydrogen in a hydrogen-oxygen fuel cell. The purification cell also provides a means of pumping and providing hydrogen in accurate quantities when controlled atmospheres are desired. The cell may also be used as a large-scale chemical processing tool.

STANLEY H. LANGER
ROBERT G. HALDEMAN
Central Research Division,
American Cyanamid Company,
Stamford, Connecticut

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Xanthine Dehydrogenase in Drosophila: Detection of Isozymes

Abstract. *Two or possibly three isozymes of the enzyme, xanthine dehydrogenase, have been detected in wild-type* Drosophila melanogaster *by starch-gel electrophoresis. Two genes, rosy and maroon-like, may control these isozymes by producing two different polypeptides that assemble in groups of three or four.*

Much evidence for the existence of enzymes in multiple molecular forms, or isozymes, has recently been accumulated (*1*). Studies of the genetic control of isozyme formation have been limited, however, by the fact that in the most well-known isozyme system, lactate dehydrogenase (LDH), only one mutant has been found among various laboratory mammals (*2*). Nevertheless, it has recently been postulated that the five electrophoretically distinct isozymes of LDH are controlled by two genes (*3*). Now it is known that at least two genes are involved in the synthesis of a different enzyme, xanthine dehydrogenase (XDH) in *Drosophila:* the rosy gene (*ry*) on the third chromosome, and the maroon-like gene (*ma-l*) on the X-chromosome (*4*). It seemed worthwhile, therefore, to determine whether isozymic forms of this enzyme exist; indications that such forms may occur were reported recently in an independent study by Keller *et al.* (*5*).

Adult wild-type *Drosophila melanogaster* (Oregon-R) were homogenized in 0.05*M* tris-buffer (*6*), *p*H 8, at a concentration of 40 mg of flies per milliliter. Norite-A was stirred in (40 mg/ml), and the mixture was allowed to stand for 30 minutes. The supernatant of this mixture, after a twofold centrifugation (30,000*g*, 30 minutes each) was subjected to electrophoresis in a starch gel made up in 0.05*M* tris-buffer, *p*H 8.7; 0.25*M* tris was used as the electrode buffer. A starch paste containing 100 to 150 μl of enzyme preparation was inserted into a slit in the gel. All these operations were performed below 5°C. Electrophoresis was continued, in the cold, for 8 hours at a voltage drop of 6 volt/cm, after which the starch gels were treated in three ways:

1) Gels were cut into 1 mm wide sections parallel to the origin. Each section was incubated in a separate test tube on a shaker in the dark at 23°C for 8 to 12 hours in an assay mixture containing tris-buffer (0.05*M*, *p*H 8, 0.4 ml), NAD (*7*) (10^{-3}*M*, 0.02 ml), and 2-amino-4-hydroxypteridine (*8*) (10^{-3}*M*, 0.02 ml). After incubation, the mixtures were centrifuged for 30 minutes at 30,000*g*, and the supernatants were placed in a Turner fluorometer, model 111, equipped with a 360 mμ primary and 405 mμ secondary filter, so that the fluorescence of the product, isoxanthopterin, could be measured.

2) A 50-μl aliquot of each of the above supernatants from each tube was applied to chromatography paper (Whatman No. 1) and subjected to ascending chromatography in the dark, in a solvent made of propanol and 1-percent ammonia in the ratio 2 : 1 (*9*). Isoxanthopterin was detected by its fluorescence and R_F by means of an ultraviolet light source emitting primarily at 360 mμ; the amount of fluorescence was estimated by eye.

3) Gels were also cut into horizontal strips, which were then incubated for 8 hours at room temperature in a histological staining mixture similar to the one used for detection of LDH isozymes (*10*), containing a saturated solution of hypoxanthine (9 ml), NAD (*7*) (10 mg/ml, 2 ml), phenazine methosulfate (1.6 mg/ml, 5 ml), nitro blue tetrazolium (*11*) (1 mg/ml, 5 ml), and tris buffer (0.2*M*, *p*H 8, 45 ml). Purple bands of formazan precipitate indicated the sites of activity of xanthine dehydrogenase in the starch.

The results in Fig. 1 show that the entire activity moved toward the anode. The graphs obtained by both the fluorometer and chromatography techniques indicate that within the first 90 mm

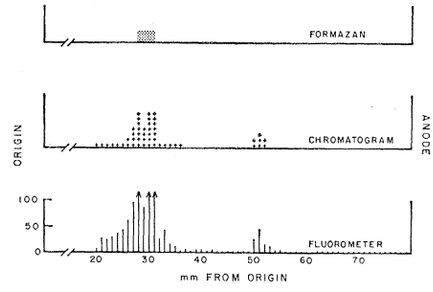

Fig. 1. Representative zymograms of xanthine dehydrogenase from wild-type *Drosophila,* obtained by three different methods as explained in the text. The number of + symbols in the middle graph reflects the estimated intensity of fluorescence. The scale on the ordinate of the lowest graph represents arbitrary units of fluorescence; arrows indicate fluorescence values exceeding the scale.

of the anodic portion of the gel, there was one large and one small peak of xanthine dehydrogenase activity. The large peak consists of two maximum points separated by a 1 mm wide zone of lower activity; whether this pair of maximum points represents different isozymes or merely double bands of a single isozyme (*12*) cannot at the present time be decided. By the formazan technique, only the large peak was detected. From our experience with LDH (*10*), the low activity of the small peak would be below the level necessary for the formation of a formazan band. Also, the narrow gap between the two maximum points of the large peak would probably not be detected by the formazan technique, since the purple band is rather faint, although clearly visible.

Assuming a subunit hypothesis similar to the one advanced for LDH (*3*), the results could be explained in two ways. If both ry^+ and $ma\text{-}l^+$ loci controlled the synthesis of a different polypeptide (henceforth called the r and the m subunit, respectively), and if these subunits then assembled in groups of four, one would expect five combinations to occur: r^4m^0, r^3m^1, r^2m^2, r^1m^3, r^0m^4. The first and the last would be enzymatically inactive (or would not be made at all), since both homozygous *ry* and *ma-l* flies lack XDH activity. Thus, three bands would be expected in this scheme. If, on the other hand, the two subunits assembled in groups of three, four combinations could occur: r^3m^0, r^2m^1, r^1m^2, r^0m^3. Again, the first and the last trimer would be enzymatically inactive (or would not be made), leaving two active bands. Our zymograms clearly show one large and one small peak. This distribution could be explained by the assumption that the two subunits are present in different amounts and that the r subunit is limiting. If the molecule were a tetramer and the large peak represents only one isozyme, then the large peak would represent the r^1m^3 tetramer, the small peak the r^2m^2 tetramer; the r^3m^1 tetramer would be produced in such small amounts as to be undetectable by our methods. If, on the other hand, the XDH molecule is a trimer, the large peak represents the r^1m^2 trimer, and the small peak the r^2m^1. In either model we assume that the r subunit is limiting. That this may indeed be so is suggested by gene dosage studies (*13*) which have shown that XDH activity increases as the number of ry^+ alleles is increased ($1\ ry^+ < 2\ ry^+ < 3\ ry^+$), but remains constant when the number of $ma\text{-}l^+$ alleles is increased ($1\ ma\text{-}l^+ = 2\ ma\text{-}l^+ = 3\ ma\text{-}l^+$). Furthermore, this shortage of r subunits may also account for the observation that offspring of $ry/+$ flies are not maternally affected, whereas offspring of $ma\text{-}l/+$ flies are (*14*).

The method presented promises to be valuable for investigating the genetic control of isozymes and for testing the proposed subunit hypothesis. However, the data obtained thus far do not rule out other models (*15*) that have been proposed (*16*).

KIRBY D. SMITH
HEINRICH URSPRUNG
THEODORE R. F. WRIGHT
Department of Biology,
Johns Hopkins University,
Baltimore 18, Maryland

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Inverted Indexing on Edge-Notched Cards

Abstract. *Edge-notched cards in an inverted system of indexing may be used satisfactorily to analyze research data which concern a small series of individuals but a large number of highly variable characteristics in a short-term research project.*

In the course of a research project on the teaching of preventive medicine in the United States, we were faced with the problem of analyzing a large mass of descriptive data from field notes and material from questionnaires, correspondence, and published references. These data could be characterized as: (i) representing a small series of individuals (medical schools); (ii) describing many characteristics or traits; (iii) possessing great diversity; (iv) not having ready-made categories into which they might be grouped satisfactorily; and (v) being part of a short-term (3-year) study and not a continuing information storage and retrieval system.

We searched for a tool which might be useful in handling these data and decided upon the use of edge-notched cards in an inverted indexing system. The purpose of this report is to discuss this method; details of the actual research project will be described in a final report (*1*). Although data-processing systems in general are discussed elsewhere (*2*), here we describe briefly the two main manual punch-card systems of indexing or grouping, and their respective physical equipment.

In the first conventional system, edge-notched cards are used (*3*) and each card represents an individual event, person, document, or other item. The coded positions around the margin of the card represent characteristics or terms, while the remaining surface of the card may be used for recording other data. Data are stored on the card by notching positions around the margin for any particular characteristic. Data are retrieved manually by passing the sorting needle through the hole in the position which represents the desired characteristic and allowing the notched cards to fall free. These cards represent all those individuals in the deck who have this particular characteristic.

In the second inverted system, peek-a-boo cards are used (*4*) and each card represents a characteristic or term. The coded positions distributed over the card represent individuals. Data are stored by punching or drilling a hole in a coded position for an individual in all the cards representing characteristics possessed by the individual. Data are retrieved by withdrawing the card representing a desired characteristic and identifying the individuals having the characteristic by visually noting the holes at the coded positions.

Since edge-notched cards do not have sufficient positions for storage of a large number of characteristics, and since conventional indexing is cumbersome in the process of developing categories requiring frequent modification, this system did not suit our requirements. Peek-a-boo cards in the inverted