able to recover the respective viruses from plants treated with gibberellic acid as readily as from untreated controls. Further studies will be directed to explain the mechanism of action of gibberellic acid in the reversal of virus-caused stunting.

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## Weber's Law and the Difference Threshold for the Velocity of a Seen Object

Although a fairly extensive literature exists concerning the visual perception of stimulus movement (*1*), only one experiment has been reported (*2*) which deals directly with the measurement of difference thresholds for the velocity of a seen object. According to that report: "An approximate correspondence with Weber's Law was found, the divergence from it appearing, in general, as an increase of the threshold at both ends of the range of initial velocities. The Mean Threshold (0.5 probability of perception, corrected for guessing) was, in favourable conditions, about 12 per cent of the initial velocity. Whether the stimulus was an increase or a decrease of velocity made no marked difference."

It is found, however, that when Hick's data are plotted (Fig. 1, broken curve), the resulting function may be interpreted as passing through an optimal $\Delta V/V$ value, rather than being a generally straight line of zero slope, which approximate correspondence with Weber's law would require. Because of this interesting alternative interpretation, a partial replication of Hick's experiment was undertaken.

Hick obtained thresholds for instantaneous increments and decrements in velocity for a pip horizontally deflected across the face of a cathode-ray tube. The total excursion of the pip seems to have been about 3.5 in., the velocity increment (or decrement) being introduced at the mid-point. For the replication, the total excursion of the pip (in inches) and the initial velocities of the pip (in inches per second) closely matched Hick's values (*2*). However, since it proved convenient to use a scope hood giving a viewing distance of 10 in., the viewing distance was approximately one-half of the 21-in. distance used by Hick. The chief consequences of this difference in viewing distance were the yield of a range of initial velocities higher than those of Hick when velocity is measured in terms of visual angle per second and the doubling, approximately, of the total angular excursion of the pip. Since Hick found no marked difference between incremental and decremental thresholds, it was decided to restrict this replication to incremental velocities only.

The remaining curves of Fig. 1 compare the incremental data obtained in the present experiment with those of Hick. In the new data, each point is based on 300 values—30 judgments for each of ten subjects; similar information is not reported in the earlier work. The

thresholds in the present study were determined by average $z$-score computations (*3*); other methods were tried and yielded similar over-all functions.

In general, the findings of the two experiments agree fairly well, showing that as initial velocity increases, $\Delta V/V$ at first decreases and then increases. Of interest is the fact that when the upper limit of initial velocities is extended, as in the present experiment, it becomes evident that if Weber's law holds at all, it does so for a very small portion of the usable range only. It may, indeed, be safe to conclude that the Weber fraction passes through a minimum in the 1-to-3-degrees-per-second region of the range of initial velocities.

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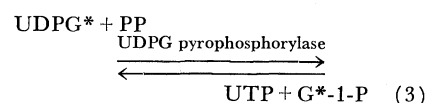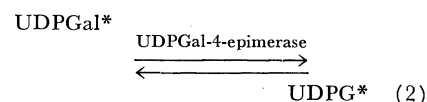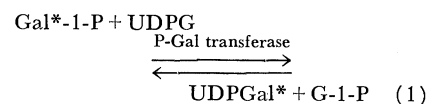*Electronics Research Laboratories,*
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## Evidence for an Accessory Pathway of Galactose Metabolism in Mammalian Liver

Recent studies on the disease galactosemia have revealed it to be the result of the congenital deficiency of a specific enzyme important in the conversion of ingested galactose to glucose derivatives (*1*). Normally, ingested galactose is first converted by means of adenosine triphosphate and galactokinase to α-galactose-1-phosphate (Gal-1-P), which can then be transformed to α-glucose-1-phosphate (G-1-P) through a series of reactions involving uridine diphosphate glucose (UDPG) and uridine diphosphate galactose (UDPGal). Thus (*2*):

$$Gal^*\text{-}1\text{-}P + UDPG \underset{\longleftarrow}{\overset{\text{P-Gal transferase}}{\longrightarrow}} UDPGal^* + G\text{-}1\text{-}P \quad (1)$$

$$UDPGal^* \underset{\longleftarrow}{\overset{\text{UDPGal-4-epimerase}}{\longrightarrow}} UDPG^* \quad (2)$$

$$UDPG^* + PP \underset{\longleftarrow}{\overset{\text{UDPG pyrophosphorylase}}{\longrightarrow}} UTP + G^*\text{-}1\text{-}P \quad (3)$$

The asterisk traces the galactose moiety through its conversion to glucose-1-phosphate. In galactosemia, the enzyme catalyzing reaction 1 (P-Gal transferase) is
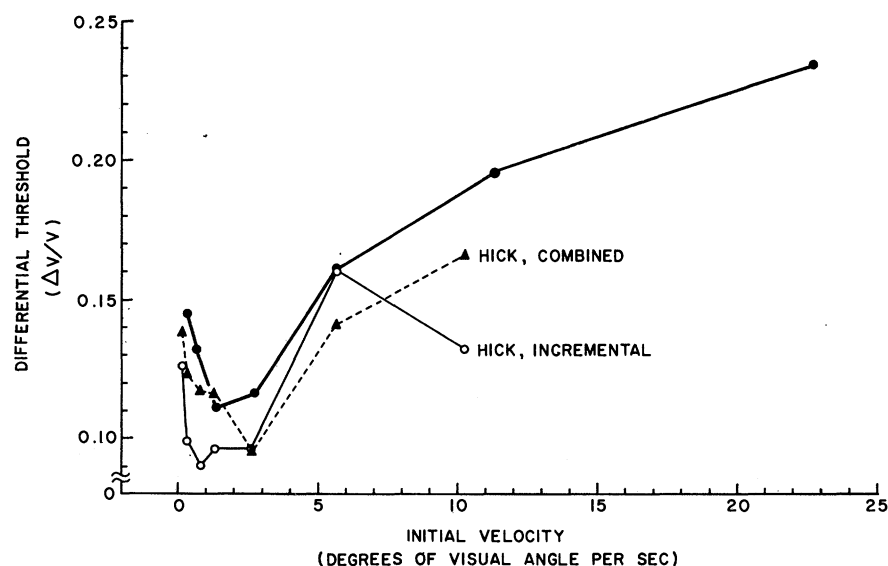


Fig. 1. Difference thresholds for the velocity of a seen object as a function of initial velocity. The uppermost curve is for data gathered in the study here reported; the other curves were plotted from tables presented by Hick (*2*).

Table 1. Incorporation of $C^{14}$-labeled α-galactose-1-phosphate into uridine nucleotide by partially purified (16-fold) rat liver enzyme.

| Incubation | Amount incorporated (count/min) |
|---|---|
| Complete system | 9,770 |
| Minus uridine triphosphate | 550 |
| Plus pyrophosphate (5 μmole) | 2,850 |
| Plus pyrophosphatase | 11,100 |
| Heated enzyme | 470 |

missing and results in the accumulation of galactose-1-phosphate. However, in spite of this demonstrated enzymatic defect, there are several indications that some metabolism of galactose is possible and does, in fact, occur in this condition. Thus, on clinical grounds it has been observed repeatedly that, as patients with galactosemia increase in age, they appear to develop an increased ability to utilize ingested galactose (3). More recently, studies on a 24-year-old male with galactosemia have demonstrated that the infusion of galactose-1-$C^{14}$ (together with oral menthol) resulted in labeling of the isolated urinary menthol glucuronosidic acid (4). From our knowledge of the role of the uridine nucleotides in glucuronosidic acid formation (5), it appeared that, in spite of the seemingly complete P-Gal transferase block, the ingested galactose was probably converted to uridine diphosphate galactose prior to the appearance of the label in the glucuronisidic acid.

These clinical and laboratory findings suggested that there might exist an additional pathway for the conversion of galactose-1-phosphate to uridine diphosphate galactose other than that catalyzed by P-Gal transferase. It seemed reasonable to assume that uridine diphosphate galactose synthesis might occur by way of a pyrophosphorolytic reaction (analogous to reaction 3) as follows:

$$\text{Gal-1-P} + \text{UTP} \xrightleftharpoons[\text{UDPGal pyrophosphorylase}]{} \text{UDPGal} + \text{PP} \quad (4)$$
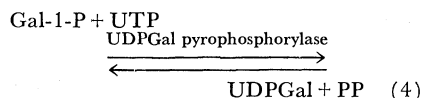
The existence of such a reaction in yeast had originally been suggested by Kalckar et al. (6), and more recently an enzyme catalyzing such a pyrophosphorolysis has been observed in plants (7).

We have now obtained evidence for the existence of such an enzyme system in mammalian liver. Rat, pigeon, and human liver were examined and found to contain enzymatic activity. The enzyme is called uridine diphosphate galactose pyrophosphorylase, in conformity with previous nomenclature. The enzyme

system was initially detected and studied by demonstrating the formation of labeled uridine diphosphate galactose from the incubation of uridine triphosphate (UTP) with 1-$C^{14}$-labeled galactose-1-phosphate and various cell-free liver fractions. The 1-$C^{14}$-labeled galactose-1-phosphate was prepared enzymatically from 1-$C^{14}$-galactose, adenosine triphosphate, and yeast galactokinase (8). The uridine diphosphate galactose formed as a result of the enzymatic reaction was identified by paper chromatography and autoradiography. It was found to migrate as a uridine diphosphate hexose in the neutral ammonium acetate-ethanol system of Leloir (9), and hydrolysis of this uridine nucleotide in 0.02$N$ $H_2SO_4$ yielded a sugar which was identified as galactose in two chromatographic systems [pyridine-ethyl acetate-water; and water-saturated phenol (10)].

By means of differential centrifugation (11), the uridine disphosphate galactose pyrophosphorylase activity has been found to be located in both the nuclear and cytoplasmic (soluble) compartments of the liver cell. From the latter fraction as well as from extracts of liver acetone powder, the enzyme has now been purified more than 16-fold and shown to be free of nucleotides, glucose-1-phosphate, and glycogen.

In Table 1 are depicted results from an experiment using this partially purified enzyme. The complete system included the enzyme (1.2 mg of protein), 0.1 μmole of 1-$C^{14}$-galactose-1-phosphate (specific activity 500,000 count/min μm), 2 μmole of uridine triphosphate, 5 μmole of $MgCl_2$, and 20 μmole of 0.2$M$ tris (hydroxymethyl) aminomethane at $p$H 7.4 in a total volume of 0.6 ml. After incubation for 30 minutes at 38°C, the mixture was deproteinized with 5-percent trichloroacetic acid. The nucleotides were then adsorbed and eluted from norite, as has been previously described (12). The radioactivity in the norite eluates shown in Table 1 represents the incorporation of 1-$C^{14}$-galactose-1-phosphate into uridine nucleotide. It will be noted that significant incorporation of counts occurred only in the complete system and that pyrophosphate inhibited, while inorganic pyrophosphatase stimulated, incorporation of the label. The reversibility of the reaction has also been confirmed by studies with C-14-uridine diphosphate galactose.

The findings presented indicate the existence in mammalian tissues of the enzyme uridine diphosphate galactose pyrophosphorylase, which provides an alternate route for both the utilization of galactose-1-phosphate and the synthesis of uridine diphosphate galactose. The enzyme has thus far been found in significant amounts only in the liver, where its activity is about one-sixth of that of P-Gal transferase (per gram of liver).

Table 2. Activity of uridyl transferase and pyrophosphorylase enzymes in rat liver with respect to age of animal. (Results are average of three sets of experiments and are expressed as millimicromoles of reactants converted per milligram of liver protein, per 20 min).

| | P-Gal transferase* | UDPG pyrophosphorylase* | UDPGal pyrophosphorylase |
|---|---|---|---|
| Fetal† | 6.8 | 340 | 0.9 |
| Neonatal (1 day) | 7.7 | 348 | 1.6 |
| Adult (60 days) | 39.4 | 98 | 7.6 |

* Determined by methods cited in (1). † The animals were sacrificed on 18th day of gestation; normal gestation, 21 days.

Additional studies on the enzymatic activity of the liver with respect to age are shown in Table 2. It will be observed that P-Gal transferase and uridine diphosphate galactose pyrophosphorylase activities are strikingly lower in fetal and neonatal rat liver than in the adult organ, while the reverse is true for the uridine diphosphate glucose pyrophosphorylase activity. In preliminary studies on human liver, similar enzyme differences with age have been found.

It is thus tempting to speculate that patients with galactosemia develop their most pronounced symptoms in infancy, because at this time they have both a pathological absence of P-Gal transferase and a physiologically feeble uridine diphosphate galactose pyrophosphorylase. The subsequent increase in activity of the latter enzyme with age might then effectively explain the improved galactose metabolism which occurs in these patients despite the continued lack of P-Gal transferase (13).

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## Anisylacetone, Synthetic Attractant for Male Melon Fly

Attractants have recently received increasing attention as an effective means for combating insect pests. By baiting traps with specific attractants, it is possible to discover an insect infestation at an early stage, and control measures may then be initiated immediately. Attractants in traps are also of use in delineating the areas that must be treated and in following the progress of the control program. Certain attractants in combination with a suitable insecticide may also be used to lure insects to their death.

The Agricultural Research Service has been conducting investigations on attractants for fruit flies at its Honolulu and Mexico City laboratories for many years (*1–3*). Since November 1955 chemists at Beltsville have been synthesizing and supplying candidate chemicals for testing in these laboratories. Recently, as a result of these studies, it was found that anisylacetone (formula Ib) is an effective attractant for the male melon fly (*Dacus cucurbitae* Coq.) (*4*).

$$R_1 - \overset{}{\underset{R_2}{\bigcirc}} - CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$$

(I)
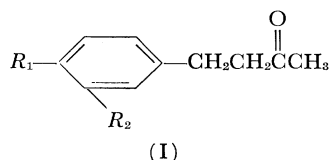
$R_1 = R_2 = H$ (Ia)
$R_1 = CH_3O; R_2 = H$ (anisylacetone) (Ib)
$R_1 = R_2 = CH_3O$ (Ic)

After more than 1000 compounds had been screened in Hawaii, it was found that the male melon fly was attracted by a number of aromatic ketones. Benzylacetone (formula Ia) and anisylacetone [formula Ib, 4-(*p*-methoxyphenyl)-2-butanone)] were the most attractive compounds, but field tests indicated that the latter was superior. These pleasant-smelling compounds are prepared by condensing an aromatic aldehyde such as anisaldehyde with acetone and hydrogenating the product. Anisylacetone has been described by Sosa (*5*), and by Chen and Barthel (*6*), who used it as an intermediate in the preparation of pyrethrinlike esters.

Analogous compounds are not as attractive as anisylacetone. Introduction of another methoxyl on the benzene ring gives a compound (formula Ic) which is no longer attractive to the melon fly but which is rather attractive to the oriental fruit fly (*Dacus dorsalis* Hendel). The similarity of this compound to the outstanding attractant for the oriental fruit fly, methyl eugenol (formula II) (*2*), is apparent.

$$CH_3O - \bigcirc - CH_2CH=CH_2$$
$$CH_3O$$

(II)
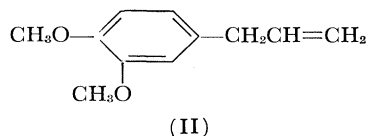
The discovery and practical evaluation of the attractiveness of anisylacetone were timely. The compound was put to use almost immediately in California to determine the extent of a possible melon fly infestation. With the help of the attractant, the state and federal officials promptly undertook an extensive trapping program, and it became apparent that the single melon fly found was an isolated specimen of unknown origin.

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8 July 1957

## Diurnal Cycles and Learning in Earthworms

Recent studies of learning in earthworms (*1*) are important, not only for the data accumulated relative to a science of comparative psychology, but also because they appear to have relevance to a general theory of behavior. Because of these considerations, as well as the general lack of basic behavioral studies concerned with this species, an attempt was made (*2*) to follow up an incidental observation of a previous study (*3*) which had indicated that the diurnal cycle of the earthworm plays a significant role in the rate at which this organism acquires a particular turning tendency in a T-maze.

An early study by Baldwin (*4*), in which observations were made of *Lumbricus terrestris* placed in dirt between two parallel glass plates $3/8$ in. apart, revealed that, in terms of crawling movements, feeding, and ejection of waste products, earthworms have definite activity cycles, with the active period occurring between 6 P.M. and 12 P.M. The present report attempts to extend these observations through study of the effect on learning of the earthworm activity cycle.

The apparatus used in this study was a T-maze constructed by fastening pieces of Lucite to a sheet of plywood. The stem and crosspiece of the maze were each 25.4 cm long, 2 cm wide, and 2 cm high; a Lucite cover was hinged in such a manner that each third of the runway could be opened independently. The left arm (negative goal) was 10.1 cm long and contained a 1-cm piece of 2/0 sandpaper 8 cm from the choice-point, followed immediately by two copper bell wires 0.25 in. apart, through which a shock of 1 v could be administered. The right arm (positive goal) was 15.3 cm long and ended in a beaker containing moist earth and moss, which was wrapped in paper in order to provide a dark interior.

One group of six earthworms (*L. terrestris*) was given five trials per day between 8 P.M. and midnight, with the negative and positive reinforcement, until a criterion of seven consecutive correct trials was achieved. The same procedure was utilized with a second group of six earthworms, except that the training was carried out between 8 A.M. and noon. A trial was considered correct if the worm entered the beaker without being shocked. Between trials on successive days, the worms were kept in a refrigerator. It should be noted that the conditions of the experimental room yielded no differential day-night cues to the two groups of worms.

Stimulation was applied by a camel's-hair paint brush if the worm remained motionless in the maze for 30 seconds. If, after 5 seconds of stimulation with the brush, the worm continued to remain motionless, a flashlight was turned on and held vertically slightly behind the anterior end of the worm. In all cases the worm began to move when the light was applied.

The mean number of trials to reach the criterion in the evening group was 32 (standard deviation, 4.01); the mean number of trials in the morning group was 45 (standard deviation, 5.27). A *t*-test shows that the evening group achieved the learning criterion in significantly ($p < 0.01$) fewer trials than did the group run in the morning hours. The

654