have similar requirements.

Adults from an inbred Oregon-R strain were isolated without etherization immediately after eclosion from stock bottles and placed in vials containing cream-of-wheat, molasses medium. After aging together for four days, they were transferred to fresh medium and eggs were collected after 30 minutes. First-instar larvae hatching over the following 23 hours were discarded, and only those hatching during the subsequent period of 1 hour were used. Salivary glands were excised in Ringer's solution precisely at respective intervals determined by the stage of maturation required. Cultures were maintained after hatching at 18°C.

As a valuable aid in reducing the variation in usual puffing pattern, two prepupal stages were recognized by specific changes in larval appearance and behavior and designated white and brown respectively. The onset of the transition of 3rd-instar larvae to the white prepupal stage was determined by the following characteristics: (1) the larval body becomes noticeably shorter and thicker, (2) the color turns from translucent to opaque white, (3) the cuticle remains flexible and soft but the surface becomes sticky, (4) locomotion and inversion-eversion movements of the anterior spiracles cease, and the spiracles remain everted. The period during which larvae remained in this stage varied from forty to sixty minutes. The brown prepupal stage followed immediately, and its onset was marked by the appearance of a faint brown pigmentation visible on the lateral edges of the cuticle. The following period of 60 (±10) minutes was characterized by the rapid darkening and hardening of the cuticle, the beginning of operculum formation, and the cessation of all movement involving the cuticle.

After dissection, salivary glands were immediately submerged in tritiated uridine (Schwartz/Mann, specific activity: 8.0 Ci/mM, 0.5 mCi, in 1.0 ml., aqueous solution) for 10 minutes, stained with aceto-lacto-orcein for 20 minutes, then prepared for analysis by the usual technique of pressure. Cover slips were removed by freezing over dry ice, autoradiographic film (Kodak AR-10) was applied to the stained slides (stripping method), the preparations were exposed for a period of three weeks, then developed and read. Photomicrographs of representative regions were taken before autoradiographic processing for comparisons with the identical regions after autoradiographic development. (Figure 1, bottom). Bridges' (1941) revised map was used to define the cytological distances. Preparations were examined at 1000X magnification, grain counts recorded, and mean grain density determined. Grain counts were taken as the mean ratio of the number of autoradiographic decay spots counted within a given puff to the mean count in a different region of the same chromosome. In general, terminal areas of the salivary chromosomes are consistently more suitable for accurate readings between bands. Thus the area between the distal end of the X-chromosome and bands 2A1-2, and between the distal end of chromosome 3L and bands 63E2-3 were used as ratio standards for counts of X- and 3L-chromosomal puffs, respectively.

Figure 1 shows several regions of labeled salivary-gland chromosomes containing puffs suitable for the procedure of counting described. Despite the higher concentration of decay spots clearly seen over the puffed regions of the chromosomes in these photographs, variation in the amount of labeling with tritiated uridine from preparation to preparation requires that grain counts of the background should be made and subtracted from counts in the region of puffs and between bands.

References: Bridges, C., 1941 (Feb.) Suppl. to J. of Hered. Vol 32, No. 2; Burdette, W. J. and M. Kobayashi, 1969 Proc. Soc. Exptl. Biol. and Med., Vol. 131.

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Improved food medium.

Our standard food medium (Mittler & Bennett, 1962 DIS 36: 131) became unsatisfactory for maintenance of a developing series of inbred and mutant lines. An experiment to modify the medium led to a replacement of the sugar content with molasses and a doubling of the yeast content. The resulting recipe: water, 1000 ml agar (powdered), 20.5 gm; brewer's yeast (powdered), 72 gm; molasses (Grandma's™ or Brer Rabbit Gold™, unsulfured), 125 ml; cook 30 minutes in autoclave at 1,055 gm/cm^2; propionic acid, 5 ml; and 1.5% benzyl bensoate in 95% ethanol, 28.5 ml, added after autoclaving. This medium is delivered to 25 x 95 mm shell vials by automatic pipette (6 ml each) and each tray is immediately cooled in cold water to prevent yeast settling. It has been used fresh and after several weeks frozen and seems equally effective in either case.