have been able to isolate similar discrete sedimenting classes of RNA from mRNA-protein particles (*14*, *19*) which are widely separated from ribosomes and polyribosomes on sucrose gradients. The size of the RNA appears to depend only on the size of the mRNA-protein particle from which it was derived. Thus a 57*S* particle contains only 33*S* RNA and a 21*S* particle contains only 11*S* RNA. Such a distribution of classes of RNA in these particles lends credence to the existence of disrete size classes of messenger RNA in the polyribosomes.

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22. The controls used to determine nonspecific binding of RNA to the membrane filters were: (i) DNA was added to the RNA immediately before filtration, (ii) RNA and DNA were incubated separately at 60° or 0°C for 24 hours and then mixed immediately before filtration, and (iii) RNA and DNA were maintained together at 0°C for 24 hours.
23. Supported by grants CA 05936-04 and CA 06927-03 from the National Institutes of Health.

29 March 1965

Bone Density Measurements in vivo: Improvement of X-ray Densitometry

Abstract. *An X-ray bone densitometer has been developed which makes direct tracings of absorption curves on a nearly linear scale. Speed and precision are increased by elimination of x-ray film. Results are reproducible to within 3 percent with a phantom finger, and within 6 percent with human subjects.*

Research to improve methods of measuring bone density in vivo continues (*1*). Our laboratory, which uses the left phalanx 5-2 for measurements, has developed an x-ray bone densitometer (*2*) to increase speed and precision in bone density determinations. Greater precision is attained by the elimination of x-ray film with its error, and by the reduction of scatter through collimation. The densitometer uses a low-intensity x-ray beam as the source of energy and utilizes a scintillation detector conjoined with a photomultiplier tube. It incorporates an electronic circuit which utilizes the logarithmic characteristic of the photomultiplier tube to produce linear absorption curves directly on graph paper by an *x-y* recorder. Figure 1 shows a block diagram of the bone densitometer. Not only is x-ray film eliminated from the procedure but approximate linearity is achieved for the absorption curves of the reference standard and test object. The instrument was calibrated at the factory with the same alloy standard used in this laboratory in the film method of bone density measurement (*3*). Daily calibrations are made with a secondary standard.

The collimated x-ray beam is located near the finger to reduce scatter and exposure to the subject. The effective radiation received at the operational setting of 40 kv and 5 ma is about 500 mr per trace. This dose is considered minimal, since it is to a narrow path of a body extremity. The x-ray tube is so shielded that radiation is negligible in the vicinity of the instrument, including operator and subject positions. The bone densitometer has an adjustable stand designed for scanning the left phalanx 5-2. The absorption curve for the chosen pathway is traced directly as the stand carries the finger through the x-ray beam. The slit size of the beam is 1 by 3 mm. The stand travels through the x-ray beam at the rate of 1 inch (2.5 cm) per 56 seconds. There are two hand positions, so that antero-posterior and lateral scans can be made. With these two scans to furnish linear measurements, the cross-sectional areas of bone and flesh used in the calculation of the bone density index are determined as ellipses with two measured axes. Figure 2 shows absorption curves of a central pathway of phalanx 5-2 as traced directly by the bone densitometer.

The bone densitometer is portable and is designed to fit into a station wagon. It operates from a 115-volt, 60-cy standard electrical outlet.

Machine reproducibility was deter-

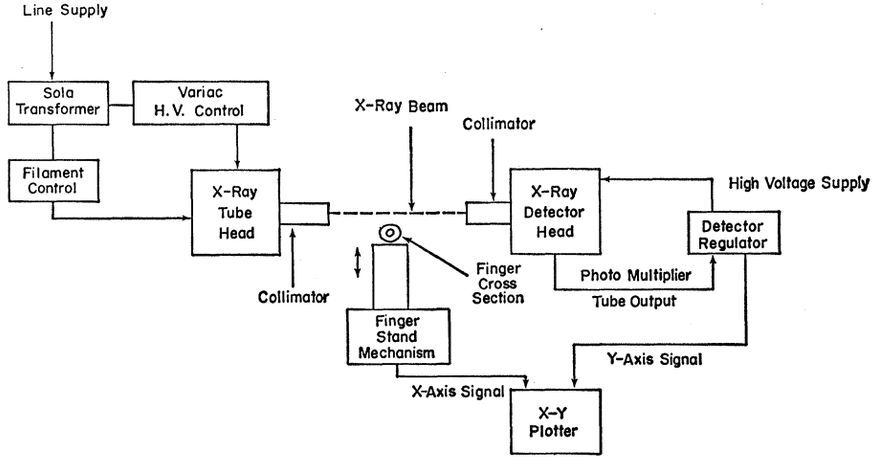

Fig. 1. Block diagram of the x-ray bone densitometer.

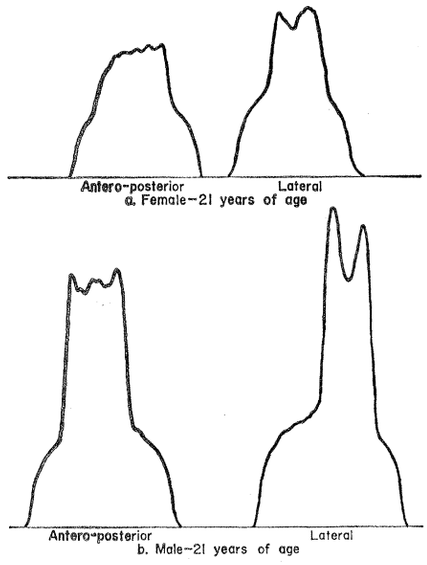

Fig. 2. Finger traces made by the bone densitometer. (*a*) Normal female, 5 feet 3½ inches (1.613 m) tall, 116 lb (53 kg) body weight, and 21 years of age, whose dietary history indicated a low calcium intake. Her present mean calcium intake is 0.55 g/day with a mean protein intake of 74.2 g/day. Her bone density index is 0.632. (*b*) Normal male, 5 feet 9 inches (1.753 m) tall, 184 lb (83 kg) body weight, and 21 years of age, whose dietary history indicated a high calcium intake. His present mean calcium intake is 2.96 g/day with a mean protein intake of 111.5 g/day. His bone density index is 1.429.

mined by making repeated measurements on a phantom finger. The phantom finger was made of a section of porcine rib encased in plastic (*4*). The plastic had a density approximating flesh density. With the phantom finger, results of the bone densitometer are reproducible within 3 percent. Repeated measurements on human subjects are reproducible within 6 percent, as compared with 12 to 14 percent when film is used.

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* Resigned.

7 June 1965

Morphogenetic Effects of 6-Azauracil and 6-Azauridine

Abstract. *When 6-azauracil and 6-azauridine are fed to larvae of the* tu^w rc *strain of* Drosophila melanogaster *which has the normal wing shape, adults hatch with shortened, obliquely truncated wings. This wing shape resembles that of the mutant* dumpy. *On the other hand, administration of these drugs to the mutant* dumpy *strain increases wing length, and flies with normal wings are obtained.*

Studies on the biological effects of azapyrimidines have been directed toward their use as bacteriostatic and cancerostatic agents. The synthesis of pyrimidine bases is inhibited by both 6-azauracil and 6-azauridine, their antimetabolic effectiveness being dependent on their conversion to 6-azauridine-5′-phosphate which affects the activity of orotidylic acid decarboxylase (*1*). Since the penetrance of melanotic tumors in tumorous strains of *Drosophila* is influenced by the amount of nucleotides and nucleosides included in the larval diet (*2*), a series of experiments was undertaken to study the effects, if any, of 6-azauracil and 6-azauridine on the development of melanotic masses in *Drosophila* larvae. During this survey phenotypic effects on wing development in *Drosophila* were noted, and these observations are summarized here.

Larvae of the *tumor^w* (*tu^w*) strain of *D. melanogaster* develop melanotic masses in the caudal fat bodies shortly before pupation (*3*). The melanotic masses survive pupal metamorphosis and are retained during adult life as inert black bodies in the abdomen. Otherwise, adults of the *tu^w* strain are morphologically comparable to the wild-type *Ore-R* strain. For our experiments, a *tu^w* stock which also contained the mutant allele *red cell* (*rc*) was used, since viability and tumor penetrance in this particular stock excel those in our stock of *tu^w*. Eggs and larvae of the *tu^w rc* stock were collected at 24°C; larvae were maintained on the usual cream-of-wheat medium for *Drosophila* until they were 67 to 68 hours of age. The larvae were then transferred to paper pulp which was moistened with the various solutions used in each experiment. Since solutions of azauracil and azauridine were prepared in distilled water, the larvae used as controls were, in each experiment, transferred to paper pulp moistened with distilled water. Larvae of *D. melanogaster* will continue development if removed from food after 65 hours of age, and normal adults, although slightly smaller in size, will emerge. A series of concentrations of 6-azauracil and 6-azauridine were tested in preliminary experiments. At a concentration of 0.5 mg/ml, 6-azauracil gives a mortality of 0.25 ± 0.034 (number of larvae, N, = 179), and 0.29 ± 0.026 ($N = 278$) of the treated *tu^w rc* larvae survive a concentration of 1 mg of 6-azauracil per milliliter. Azauridine equimolar with the former concentration of azauracil produces a mortality of 0.12 ± 0.017 ($N = 347$).

The wing shape of *tu^w rc* adults is the same as that of the wild-type *Ore-R* strain, and Fig. 1*A* demonstrates the appearance of the wing of a *tu^w rc* control specimen transferred to H_2O when the adults are 67 hours of age. After the feeding of azauracil or azauridine, larvae were obtained with an oblique shortening of the wing (Fig. 1*B*). This wing shape closely approximates that of the known mutant, *dumpy* (*dp*) (Fig. 1*C*). Various degrees of this effect are obtained, some wings showing a shortening as extreme as that of the *dp* mutant and other wings displaying a truncation of the 1st and 2nd posterior cells with the wing appearing pointed. Both wings of a fly are affected to a similar degree, and only an occasional disparity between the wings of an affected individual is noted. After treatment with azauridine (1 mg/ml), 23 percent of the individuals hatching ($N = 238$) showed effects on the wing; 44 percent of the hatching adults ($N = 57$) showed wing abnormalities after administration of 0.5 mg of 6-azauracil per milliliter; and 83 percent of the surviving individuals ($N = 24$) were affected by feeding 1 mg of 6-azauracil per milliliter.

The term phenocopy designates an artificial phenotypic effect which resembles the phenotypic expression of a known mutant. The morphological abnormality produced by azauracil and azauridine resembles that of the mutant phenotype of *dp*. However, the underlying mechanism in phenocopy production may not be the same as that involved in the action of the mutant gene *dp*. If the mechanism of phenocopy production parallels the action of the mutant gene, treatment of the mutant strain with these chemicals would