the solution of insoluble calcium salts is favored in the presence of this anion (*8*). From experiments on the equilibration of apatite systems and of powdered bone with buffered citrate, it is evident that soluble (and insoluble) complexes are formed with bone mineral (*5, 6, 9*). (ii) Citrate occupies a position of central importance in the scheme of cellular metabolism, and bone contains the enzyme systems necessary for the synthesis and utilization of this compound (*2, 4*). (iii) Citrate occurs in relatively high concentration in the extracellular matrix of bone (*10*). (iv) An elevation of serum citrate levels often accompanies bone resorption (*11, 12*).

In testing our theory, we studied soluble citrate concentrations in medullary bone of the pigeon during the egg-laying cycle (*13*). At such times, rapid transformations occur in the tibia and femur (*14*). For approximately 1 week preceding calcification of the egg, a period of bone apposition is dominant. Then during calcification of the egg (a clutch of two eggs is laid within 40 hours) resorption predominates, and the serum citrate level is elevated (*12*). In the third stage, this cyclic activity is discontinued and bone apposition and resorption are minimal. The soluble citrate concentrations were determined during these three stages.

Previous efforts to demonstrate local changes in citrate concentration in bone have been clouded by the inclusion of a large quantity of citrate from the insoluble extracellular phase (*15*). We have attempted to overcome this difficulty by a procedure which largely sequesters cells and soluble extracellular material. Hence, our results measure intracellular citrate plus any liberated soluble extracellular fraction.

Thirty pairs of mated pigeons were used for these experiments. The birds were examined daily until their egg-laying cycle was determined. They were then sacrificed during the resting, apposition, or resorption period. The exact period was confirmed by gross examination of the ovaries and ovulatory tract and by gross and histologic examination of the long bones. Material for citrate determination was obtained from the tibia and femur. For this procedure the bones were excised and split longitudinally, and the cells in the medullary portion were aspirated through a fine-bore (1 mm) glass tube into a chilled flask containing a small amount of distilled water. A piece of 100-mesh wire screen, moistened with water, was interposed between the bone and the tip of the tube to prevent aspiration of bone particles. The surface of the bone was continually shaved with a razor blade to expose fresh areas of cells. The aspirated material was homogenized and then lyophilized.

Table 1. Soluble citrate aspirated from pigeon medullary bone.

No. of birds	Stage	Citric acid* (μg/g)	*P*
8	Resting	167 ± 82	
7	Apposition	846 ± 203	< .001
15	Resorption	1361 ± 304	

* Mean values and standard deviations expressed as micrograms per gram of lyophilized material.

Fifty-milligram portions of the dried material were analyzed for citric acid by L'Heureux and Roth's modification of the method of Natelson, Pincus, and Natavoy (*11*).

The results, expressed as micrograms of citric acid per gram of dry aspirated material, are given in Table 1. During the resting phase the citrate level was 167 μg/g. This level increased about five times during the stage of bone apposition. The highest concentration, representing an eightfold increase over the resting level, occurred in the resorptive stage.

These values may be compared with the citrate concentration in the serum (approximately 5.7 mg/ml) observed during the resting stage (*12*). To express the data in terms of tissue water, we will assume that the extracted cellular material has a water content of 80 percent. Then the calculation yields the following values: resting stage, 4.2 mg/100 ml; appositional stage, 21.2 mg/100 ml; and resorptive stage, 34.0 mg/100 ml. Thus, the concentration of citrate from the bone sites during the cycle exceeds that in serum from resting birds by approximately 4 to 6 times. We have no information about the rate of formation and utilization of citrate by the bone; nevertheless, this high local citrate concentration alone would be expected to have an important effect on the state of the bone salts and bone matrix.

In in vitro experiments with calcium phosphate, apatite, and bone powder, it has been shown that citrate can react with calcium to form both soluble and insoluble compounds (*6, 9*). When a small amount of citrate is present, it is coprecipitated with calcium phosphate (or apatite). On the other hand, larger concentrations lead to the formation of soluble calcium citrate and to solubilization of the altered apatite. Although the nature and relationships of many of the components and phases of bone are still ill defined, we assume that in bone, citrate is also distributed between an insoluble phase in the calcified matrix and a soluble phase, as a calcium complex. This concept, in conjunction with our present and earlier experimental observations, leads to the following hypothesis concerning the role of citrate in bone apposition and resorption. During bone formation, the citrate released through cellular metabolism is largely deposited in the insoluble solid phase. As citrate production continues and the concentration increases, the soluble calcium citrate complex is formed, removing calcium from the ionic, crystalline, and protein bound fractions. Accompanying this realignment in the phase relations there is a disaggregation of the structure of bone. As a corollary of this view, the role of citrate in cell metabolism and its equally significant part in bone apposition and resorption illustrates the unity of structure and function.

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Display of Moving Parts of a Scene

Abstract. Methods for emphasizing the moving parts of a scene. By photographic or electronic means, a past image can be subtracted from the present one to emphasize the moving parts of a motion picture scene. Rhythms and patterns of motion become more noticeable, and changes in velocity can give an impression of accelerations and the force pattern.

In a motion picture, it is possible to emphasize those parts of a scene that move by suitable use of photographic or electronic methods. In essence, the method compares the image at one instant with that at a slightly later in-

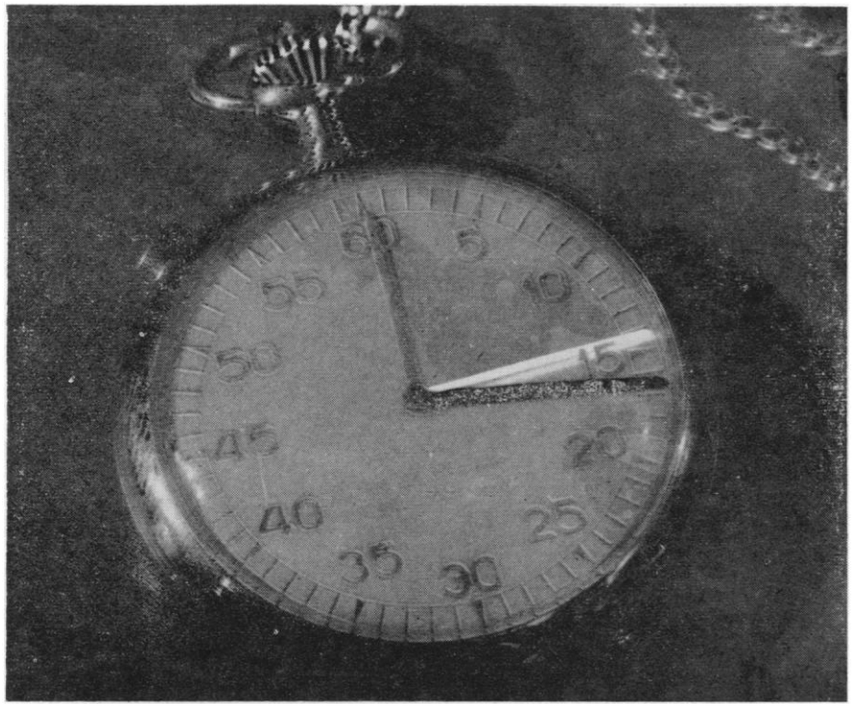

Fig. 1. Enlargement of a 16-mm motion-picture frame showing typical effect of sequential double printing. The stationary structure is deemphasized. Specular reflections from the watch stem and chain result in nonlinearity and less complete cancellation in those areas. The effect is more impressive when the pictures are projected in motion.

stant and displays only the differences between the two. The rhythms and patterns of motion can thus be made more noticeable. Applications include the study of motion in general and especially that propagated as waves, but my special interest is in speech and motion studies in connection with x-ray movies.

If a motion picture is to be studied in this fashion, one first makes a negative print from it, developed to a gamma of unity. The print and the original are then shifted a few frames relative to each other and projected together. The bright patterns of the stationary parts of the scene on the original will have superimposed upon them the dark patterns of the print. Within the linear portion of the H and D curve, stationary parts of the scene will thus register as a relatively uniform grey. Only those parts that do not coincide fail to cancel. The amount of relative shift used is chosen by taking into account the speed of the motion of interest, the stability of the projector, and the fineness of detail in the scene. For example, a film of a clock face projected with a shift of 20 frames relative to the negative will show the second hand moving, the other hands and the face being almost uniformly grey. Register and processing are important in creating the uniform background. Imperfection in either will allow the background to show up dimly, but this is convenient for orientation. In general, the purpose of the display is to outline objects that move and to emphasize brightness gradients in the subject that shift; fine details may appear double.

If no projector suitable for showing two films at once is available, a single print can be made from a positive and negative of the original. This print can then be developed to high gamma. In using an ordinary movie printer, one must make the final print by printing from two films of identical gamma, both printed from the original and one having been developed as a direct positive and the other as a negative. The positive and the negative can then be printed one after the other onto the final film, and in both runs there will be contact between emulsions for good focus. A very light or dark object will print through, since a transparent region in one film cannot be cancelled by the corresponding opaque region on the other film. However, if one can stay in the linear region and avoid saturation of the film, then it will not be necessary to print from both films at once in a special optical printer. For then the final exposure is the sum of its two parts, and extra light from one film will be compensated for by less light from the other. Simultaneous printing is desirable not only because one less intermediate film is required but also because a greater brightness range can be handled, due to the exposure of the final film to differences only.

Figure 1 shows a subject in which there were strong contrasts; the film was processed sequentially. Attention is attracted to the faster-moving watch hand; the other one is less noticeable. Despite the wide range of brightness in the subject, extreme care was not needed to obtain this degree of cancellation. The large frame shift produced considerable doubling. The sense of motion, if not previously known, would be indicated by the dark component's leading the light one (though the effect would be reversed if the background were dark or if the opposite film shift had been employed).

A television magnetic tape recorder can be used to provide the same result in two ways. Information stored on a video tape can be immediately displayed in this fashion if a double pick-up head is used, the two outputs feeding a difference amplifier. Alternatively, any incoming image can be directly converted to this form by splitting the signal along two paths, one going toward the output device and one to the recording head of a video recorder. The read-out head farther along "plays" into the output device, which contains a difference amplifier, and thus displays the difference between the signal at this stage and the signal at a fixed previous time. There is an erase head after the read-out head, and thus a continuous loop of tape may be used. In this mode of operation the tape recorder functions as a delay line; any other memory device or circuit could function similarly if it had sufficient storage capacity and speed. The noise level in presently available video tape recorders is rather objectionable in this application. However, a radiologist, if he is not administering an excess dose, is accustomed to fluctuations or noise of this magnitude, and, therefore, in x-ray applications a tape recorder may prove acceptable. The effect of noise in the recorder can be reduced by repeating each scene several times in a period that is much shorter than that of the original action.

The foregoing discussion pertains to moving pictures and extended observation, but this general method can be applied to any pair of images, and it then does not necessarily involve more than two ordinary pictures. One can use color in a similar way by dyeing two films different colors and forming a final color print (*1*).

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Note

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