A Simple Ink Writer for the Mercury Manometer

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Methods have been described for recording, in ink, carotid and femoral blood pressures in experimental animals, employing the mercury manometer (*1*, *2*). These methods involve rather complex apparatus.

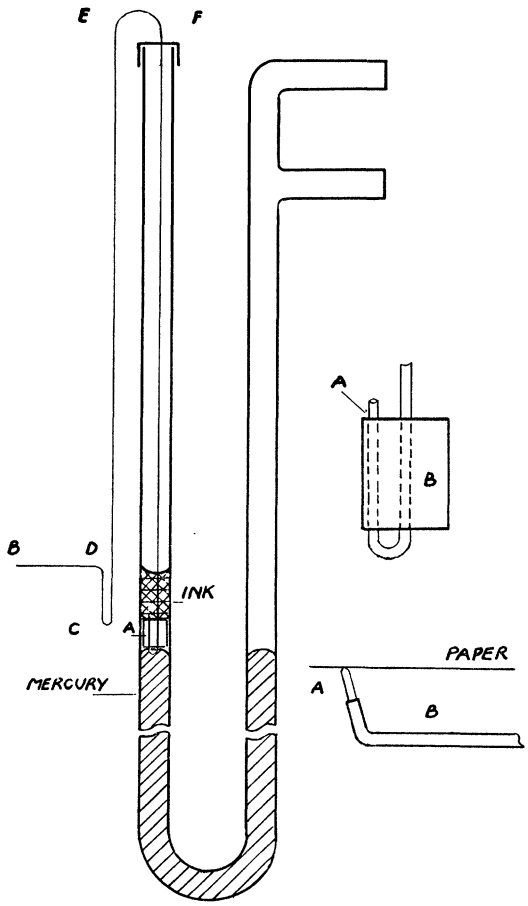

FIG. 1. Mercury manometer with ink writer in operating position. Illustrations at right show (top) detail of attachment of tubing to float (A, open end of tubing; B, solid Bakelite float) and (bottom) writing point viewed from above (A, 27-gage, and B, 22-gage stainless-steel tubing).

The ink writer now in use in this laboratory consists of a length of 22-gage stainless-steel tubing,[1] one end of which is attached to a float (A) and dips into a reservoir of ink floating on the mercury. The other end of the tubing serves as the writing point (B). Ink is drawn over to the writing point by siphonage.

[1] Obtained from Becton, Dickinson and Co., Rutherford, New Jersey.

The ink writer illustrated (Fig. 1) is approximately 20 cm in height. The writing point is 27-gage tubing, rounded on the end and inserted into the lumen of the 22-gage tubing. The float, a solid Bakelite cylinder 6 mm in diameter, fits the bore of the glass manometer, the cap of which is shown at F. Bends in the tubing (C and D) permit the writing point to be slightly above the level of ink in the reservoir, so that the ink is transferred to the kymograph paper by capillary action.

A small 'V' guide, clamped to the manometer support, eliminates lateral motion in the writing point due to vibration. The 'V' so rests against the tubing that it holds the writing point against the kymograph paper and at the same time allows unrestricted vertical excursions.

Prior to use, the writer is filled with ink from a hypodermic syringe by fitting a 27-gage needle hub, from which the shaft has been removed, over the end of the writing point.

Ink writers for signal magnets are constructed from 22-gage hypodermic needles, bent so that the hub serves as the ink reservoir. The needle hub is held by a spring clip soldered to the spring bar of the signal magnet.

References

1. FEITELBERG, S. *Proc. Soc. exp. Biol. Med.*, 1942, **49**, 177.
2. QUIGLEY, J. P. *J. lab. clin. Med.*, 1934, **19**, 1342.

The Demonstration of P^{32} in Bone by Radioautography

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The study of phosphorus metabolism in bone by radioautography, using P^{32}, has been limited by the technical difficulty involved in obtaining undistorted thin sections of undecalcified bone. Axelrod (*1*), who noted the removal and shifting of radioactive material during the process of decalcification, resulting in unsatisfactory or inaccurate autographs, developed a technique to cut 6- to 10-μ sections of celloidin-embedded undecalcified rat bones. The writer, using her technique, obtained satisfactory autographs in rats, but the histological detail of the stained sections was quite distorted. When the method was tried in rabbits, the bone proved to be too hard to cut with the microtome-blade technique.

A method was sought, therefore, to prepare bones of larger animals, containing radioactive phosphorus, for radioautographic studies. The possibility was investigated of using relatively large amounts of radioactive material as a tracer dose and partly decalcifying bone to the extent that thin, undistorted sections could be obtained, both for radioautographic and histological study. Using the inorganic acids usually employed in the decal-

[1] The author expresses his gratitude to Dr. Sergei Feitelberg, director, Department of Physics, for his invaluable suggestions and discussions and to Dr. Robert K. Lippmann, director, Orthopedic Surgery, under whose auspices this work was undertaken.

cification of bone (nitric and hydrochloric), it was noted that by the time bone was soft enough for easy cutting, most of the radioactive material had been removed. With formic acid, however, rapid bone softening was obtained (2 hrs in 2- to 3-kg growing rabbits using 30% formic acid), and relatively much larger amounts of radioactive element could be demonstrated in the bone.

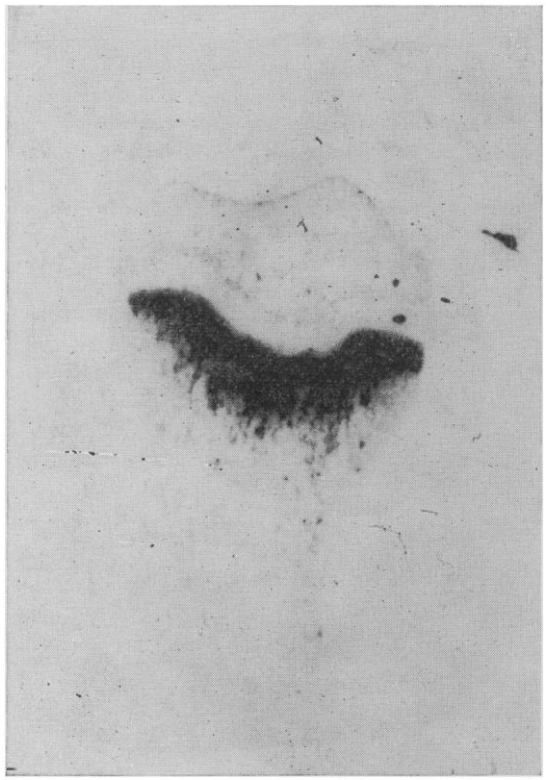

FIG. 1. Radioautograph of lower end of radius of growing rabbit demonstrating how P^{32} is metabolized in the epiphysis, epiphyseal line, and metaphysis (10× magnification; from 8-μ section).

The principle of binding phosphates at the site of liberation from organic compounds was introduced by Gomori (*3*) in the demonstration of acid and alkaline phosphatase. In the former reaction, inorganic phosphates are liberated from organic sodium glycerophosphate substrate by the action of the enzyme phosphatase in the tissue sections in an acid medium. The liberated inorganic phosphate is then immediately precipitated in the tissues at the site of enzyme activity by lead ions in solution, in the formation of insoluble lead phosphate. Although the softening of bone with acids is a far more complex reaction than the breakdown of glycerophosphates, this concept was applied to the decalcification process. It was felt that the presence of lead ions might bind phosphates as insoluble salts at their site of liberation from the complex bone apatite molecule during decalcification. By this means, larger quantities of P^{32} might be retained in bone that had been sufficiently softened so that thin sections could be easily cut in the preparation of detailed histological sections and radioautographs.[2]

Growing rabbits of 1.0 to 2.5 kg were given intravenously 0.5 and 1.2 mc of P^{32}, respectively, and killed the following day. The bone was fixed in formalin to which a small amount of lead acetate (1–2%) had been added. It was found advisable to suspend the bone in

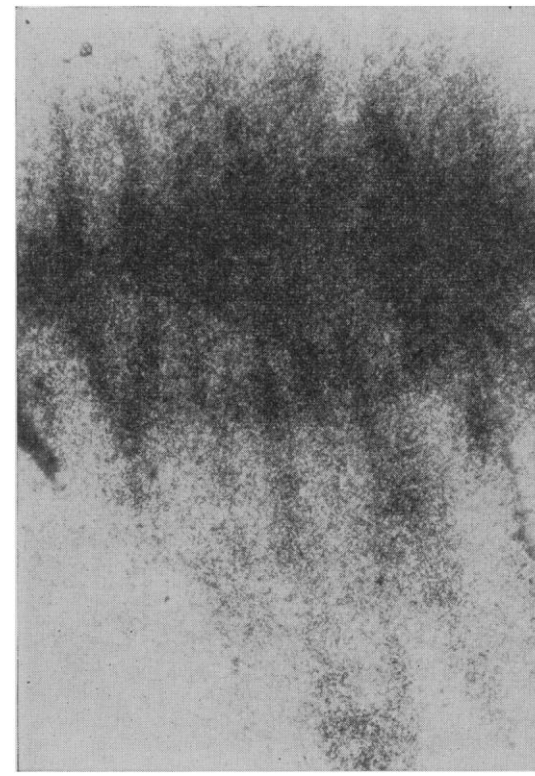

FIG. 2. Radioautograph of epiphyseal line demonstrating excellent detail of deposition of P^{32} along the pattern of the growth columns (100× magnification; from 8-μ section).

gauze in the solution for several days to prevent the deposition of insoluble lead salts on the surface of the bone and to permit uniform adsorption. Bone was then washed thoroughly and decalcified by suspension in gauze in a solution of 30% formic acid until soft by the needle test (2 hrs). This could be hastened by decalcifying in the incubator at 37° C. The specimen was then washed thoroughly, embedded in paraffin, and cut at the desired thickness. The sections (8–30 μ) were cut with ease with no niching of the blade. Bone oversaturated with lead proved to be rather hard and crumbled on cutting. Experience was required to determine that point at which bone was soft enough for easy cutting, without permitting overdecalcification. Radioautographs were made after mounting the sections on slides and press-

[2] Grateful acknowledgment is made to Dr. Boris Gueft, Department of Pathology, for his suggestion that this principle of binding phosphates with metallic ions be applied to the present problem.

ing the dried sections, or sections coated with collodion, against a high-speed, no-screen, X-ray film (dental) in the dark. Sections cut alternately with those used for autography were mounted and stained directly for histological study. Excellent detailed radioautographs and histological sections were obtained and could be easily compared (Figs. 1–3).

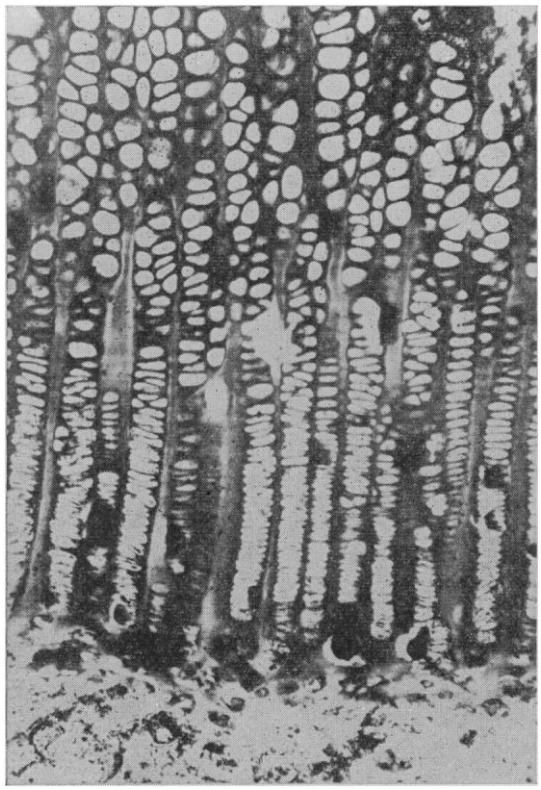

FIG. 3. Photomicrograph of epiphyseal line demonstrating undistorted histological detail (200× magnification; 8-μ section).

It was noted that the autographs at 8 μ, when viewed under the 100× power of the microscope, were slightly finer in detail, with less scatter than those obtained from the 30-μ sections. Detailed and gross radioautographs prepared by this method of partial decalcification, with and without the use of lead, revealed that although large amounts of P^{32} were removed, consistently much larger quantities were retained in those specimens in which lead was used. Further investigation is indicated to determine the nature of the lead-phosphorus combination. A more careful control of chemical conditions, and perhaps the use of other metallic substances, might increase the amount of phosphorus bound as insoluble salt at the site of liberation.

When nitric or hydrochloric acids were used in place of formic acid, the amount of phosphorus retained after partial decalcification using lead was not greater than in those specimens where lead was not used. When acetic acid was used, decalcification was very slow, often requiring 2 weeks. In spite of this prolonged period, excellent detailed autographs were obtained only if the lead technique was used. It appears, therefore, that the lead-phosphorus compound formed is soluble in nitric and hydrochloric acids and insoluble in formic and acetic acids. The success of the method, then, probably depends upon the formation of metallic salts, with the liberated phosphate compounds during decalcification, that are insoluble in the solutions used for decalcification. The optimum pH is yet to be determined.

The possibility of transfer of radioactive material to nonradioactive areas during decalcification was considered. Comparative adsorption studies of P^{32} in solution into nonradioactive bone were performed by the use of autographs and the Geiger counter. It was noted that adsorption by bone was consistently much less from a 30% formic acid solution than from a water solution of P^{32}. The addition of lead to the solution before the bone was immersed resulted in the precipitation of an insoluble lead phosphate salt, permitting only relatively little adsorption of phosphorus into the suspended bone. From the beginning of decalcification through radioautography, pieces of rabbit bone containing no radioactive element were processed at the same time and in the same solutions as the pieces containing P^{32} and lead. Final radioautographs never demonstrated a shift of radioactive material to the control bone, regardless of whether the latter had or had not been presoaked in a lead acetate solution. It was felt that this, in addition to the fact that the autographs obtained showed the same distribution of P^{32} in 2.5-kg growing rabbit bones as did those prepared from undecalcified rat bones by the Axelrod technique, is further evidence that there is no material shift of the radioactive element. Geiger counter studies on the control specimens always revealed the adsorption of small amounts of phosphorus, but apparently the quantity of shift was so small compared to the quantity retained that it could not be demonstrated by autography. It may be that the presence of lead ions in solution causes the formation of an insoluble lead phosphate with those phosphate ions that are not locally bound on liberation, preventing readsorption into nonradioactive areas.

Since much of the radioactive material is removed by this method of partial decalcification, even in the presence of lead ions, it is necessary to use relatively larger tracer doses. In order to be sure that material is not completely removed from critical areas under study, a preliminary profile is recommended. The type of profile will depend upon the character of any particular experiment and the detail required. Counts of calibrated areas before and after decalcification using a small hole in a lead shield, or a control autograph on an undecalcified section polished to 1- to 200-μ thickness (*2*), could be performed. Further investigation is indicated to determine whether, under optimum circumstances, sufficient amounts of radioactive element are removed to require control profiles.

In vivo studies performed by injecting 200 mg of lead acetate intramuscularly daily for 3 successive days into 2.5-kg growing rabbits, followed by the injection of

0.5 mc of P^{32}, were performed. Bone was partially decalcified with formic and nitric acids. Those sections decalcified with formic acid gave satisfactory autographs and demonstrated the retention of lead when dipped into a dilute solution of yellow ammonium sulfide. Those decalcified with nitric acid retained neither P^{32} nor lead.

Preliminary observations on adult giant rabbit and dog bones, softened overnight in formic acid, indicate that, since the metabolic turnover of phosphorus is slower than in growing animals, much larger tracer doses and longer intervals between injection and sacrifice of the animals are required. Further investigation is required to determine whether the method is applicable to adult rabbits and dogs.

References

1. AXELROD, D. J. *Anat. Rec.*, 1947, **98**, 19.
2. BARTELSTONE, H. J., MANDEL, I. D., OSHRY, E., and SEIDLIN, S. M. *Science*, 1947, **106**, 130.
3. GOMORI, G. *Arch. Path.*, 1941, **32**, 189.

Lipemic Nephrosis in Rats[1]

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The experimental production of chronic renal disease by means of heteronephrotoxins was first reported by Lindemann (*3*). Masugi produced the disease in rabbits and described it as chronic glomerulonephritis (*4*). Smadel, Swift, and Farr (*7*) studied nephritis in rats induced by intravenous injection of antikidney serum obtained from rabbits and described it as a diffuse chronic and progressive glomerulonephritis. Smadel and Swift (*5, 6*), using rats of the Whelan, Evans, and Wistar strains, observed decreasing susceptibility to nephrotoxin and increasing capacity to recover from the initial nephrotoxic injury, in that order. Progressive glomerular disease was more severe in the Whelan than in the Evans and Wistar rats, and a high protein diet aggravated the course in the former and not in the latter strains (*7*).

We used the technic described by Smadel, Swift, and Farr with the following differences: (a) We used a Waring blendor for the preparation of kidney extracts; (b) we kept extracts and sera frozen for indefinite periods; (c) after addition of 1,000 units of penicillin/cc, some of our extracts were kept under toluene for 24 hrs at 37° C in an incubator; (d) the amount of serum given was not based on body weight but was calculated according to kidney weight; (e) we used rats of the Long-Evans strain exclusively; and (f) some rabbits were injected intramuscularly with extracts incorporated into an emulsion containing paraffin oil, a lanolin-like substance, and dry heat-killed tubercle bacilli (*1*).

[1] Aided by grants from the Life Insurance Medical Research Fund, the John and Mary R. Markle Foundation, and the Louis D. Beaumont Trust.

Sixteen rabbits were repeatedly injected intraperitoneally and 3 intramuscularly with renal extracts obtained from rats. The intraperitoneal treatment has thus far been more efficacious in producing nephrotoxic sera than has the intramuscular injection of extracts incorporated in Freund's adjuvants.

Six rabbits were treated with renal cortex, 7 with renal medulla, and 6 with extracts obtained from undissected kidneys. The separation of cortex and medulla was approximate and was done by sharp dissection. All of the sera obtained from rabbits treated with cortex and whole kidney extracts produced chronic renal disease in rats. Among the 6 sera obtained from rabbits treated with renal medulla, only 1 was about equally nephrotoxic, 2 produced a mild, transient proteinuria, and 3 were inactive. The ability of medulla extracts to produce nephrotoxic sera was not enhanced by injecting rabbits with a mixture of 2 parts of a 20% medulla suspension with 1 part of a 20% rat spleen suspension.

Renal disease produced by intravenous injection of the various nephrotoxic sera was obtained in 103 rats. The production of disease depended on dosage and on the individual susceptibility of the rats. Massive proteinuria was observed 1–2 days after intravenous injection of a nephrotoxic serum. When boiled with acetic acid, their urine often coagulated. Gross hematuria was not observed, and microscopic erythrocyturia was rare. A few leucocytes and numerous casts were usually present. Within the first and second week marked ascites and edema developed in 33 animals and persisted usually for 1–3 weeks. The natural course of the disease was observed in 35 rats. Spontaneous cures were observed in 13, a succession of remissions and relapses was seen in 12, and 10 rats showed a continuous, uninterrupted proteinuria for as long as 11 months. Blood pressure readings obtained in 42 unheated, nonanesthetized animals (*2*) varied between 90 and 125 mm Hg.[2] Forty animals remained normotensive during the course of their illness. In 2 rats hypertensive episodes with systolic values ranging between 130 and 145 mm Hg were observed $3\frac{1}{2}$ and $7\frac{1}{2}$ months, respectively, after onset of their disease.

Severe hypoproteinemia (lowest value, 1.6 gm/100 cc) and marked hyperlipemia (highest values, 1.96 gm/100 cc cholesterol and 19.5 gm/100 total lipids) were regularly observed in severely sick animals. In late stages of the disease a moderate degree of azotemia was observed only once. However, high nonprotein nitrogen values (100–200 mg/100 cc) were frequently obtained when rats were injected with lethal doses of markedly nephrotoxic sera. The blood pressure in all these animals was normal, and the highest concentration of creatinine observed in them was 2.6 mg%. High (40%) or low (5%) protein diets, otherwise isocaloric, were without influence on course or severity of the disease.

Histological examinations of kidneys and other tissues were obtained in 83 rats. The conspicuous renal change

[2] An apparatus was obtained from the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of the late Y. SubbaRow.