

for each form, although there are some, like the armadillo, which give equally good results after almost any period of fixation.

In some forms which have yolky eggs, fixation tends to harden the yolk until it is almost impossible to section satisfactorily. In such cases dilution of the fixative with an equal amount of water may give satisfactory results. This procedure has proved successful with fish eggs and reptile eggs, after ordinary methods had failed completely, and it gives more consistent results with amphibian ova than does the full strength fixative. Mitotic figures in embryos are as distinct and well-fixed following the dilute Bouin's as they are when preserved in the full-strength fluid.

DEHYDRATION

Even after proper fixation, it may prove difficult to get good sections. Many difficulties have been blamed upon the hardening effects of xylol and the paraffin oven. I have come to realize that neither xylol nor paraffin of any reasonable temperature can do any damage comparable to that caused by alcohols of 80 per cent. or higher. To avoid the use of these alcohols I substitute anilin oil as a dehydrating agent. The procedure used is as follows.

Material fixed in Bouin's—35 per cent. alcohol—50 per cent. until excess picric acid is removed— $\frac{1}{3}$ anilin + $\frac{2}{3}$ 70 per cent.— $\frac{2}{3}$ anilin + $\frac{1}{3}$ 95 per cent.—pure anilin until tissue is completely cleared— $\frac{1}{2}$ anilin + $\frac{1}{2}$ xylol (or use more gradual steps if material shows

tendency to shrinkage)—xylol at least an hour—xylol + paraffin—paraffin.

I have left material, treated in this way, over night in xylol, and in a paraffin oven for twelve hours at 58°, without making it brittle or hard. The same material, run through absolute alcohol, would have shattered to pieces or turned the edge of the microtome knife. Using this procedure, I can get perfect serial sections of 10 mm mammalian embryos *in situ* within the unopened uterus. Following alcohol, material of this size would become impossibly hard long before infiltration was complete. Similarly, the yolk-laden eggs of teleost and lizard remain soft and workable when the anilin method is used.

There is another advantage of the anilin method that is important in some cases. Tissues become quite tough in $\frac{2}{3}$ anilin and anilin, and at the same time clear much more completely than with xylol. It is accordingly possible to carry out delicate dissections with ease and at the same time with little risk of accidentally injuring the tissues.

The danger-point in the process is in the transfer from anilin to xylol. Even with one or two intermediate steps the diffusion currents set up are so strong that blastocysts or thin-walled cavities of any sort are likely to collapse partially. The remedy lies, of course, in making the transition more gradual, if necessary running in the xylol by the drop method.

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SPECIAL ARTICLES

THE APPARENT EXISTENCE OF EASILY DEFLECTABLE POSITIVES

Up to the present a positive electron has always been found with an associated mass 1,850 times that associated with the negative electron. In measuring the energies of charged particles produced by cosmic rays some tracks have recently been found which seem to be produced by positive particles, but if so the masses of these particles must be small compared to the mass of the proton. The evidence for this statement is found in several photographs, three of which are discussed below.

In one instance, in which a lead plate of 6 mm thickness was inserted in the cloud-chamber, tracks of a particle were observed above and below the lead. The curvature due to the magnetic field was measurable both above and below the lead. There are the following alternative interpretations:

(1) a positive particle of small mass penetrates the lead plate and loses about two thirds of its energy; or

(2) two particles are simultaneously ejected from

the lead, in one direction a positive particle of small mass, in the opposite direction an electron; or

(3) an electron of about 20,000,000 volts energy penetrates the lead plate and emerges with an energy of 60,000,000 volts, having gained 40,000,000 volts energy in traversing the lead; or

(4) the chance occurrence of two independent electron tracks in the chamber, so placed as to give the appearance of one particle traversing the lead plate.

In another instance two tracks of opposite curvature appear below the lead. The alternative interpretations are:

(1) a positive particle of small mass and an electron emerging from the same point in the lead; or

(2) a positive particle of small mass strikes the lead and rebounds with a loss in energy; or

(3) an electron of about 20,000,000 volts energy strikes the lead and rebounds with 30,000,000 volts energy; or

(4) the chance occurrence of two independent electron tracks.

In the third instance two tracks appear below the lead plate. The alternative interpretations are:

(1) a positive particle of small mass and another positive particle emerge from the same point in the lead; or

(2) a 4,000,000 volt electron rebounds from the lead producing the second track; but here a difficulty is met with, since a change in the sign of the charge would have to be assumed to take place in the rebound of the electron; or

(3) the chance occurrence of two independent tracks.

For the interpretation of these effects it seems necessary to call upon a positively charged particle having a mass comparable with that of an electron, or else admit the chance occurrence of independent tracks on the same photograph so placed as to indicate a common point of origin of two particles. The latter possibility on a probability basis is exceedingly unlikely.

The interpretation of these tracks as due to protons, or other heavier nuclei, is ruled out on the basis of range and curvature. Protons or heavier nuclei of the observed curvatures could not have ranges as great as those observed. The specific-ionization is close to that for an electron of the same curvature, hence indicating a positively-charged particle comparable in mass and magnitude of charge with an electron.

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PLASMA PHOSPHATASE IN DAIRY COWS SUFFERING FROM FLUOROSIS

FLUORINE, when included in experimental diets of animals, has been shown to cause a marked disturbance in bone and tooth metabolism. Robison¹ in 1923 showed that an enzyme capable of splitting phosphoric acid esters was instrumental in bone deposition. Kay² in 1930 brought forth evidence to show that the plasma phosphatase increased in such bone diseases as rickets and osteomalacia. The possibility of a change in plasma phosphatase in chronic fluorine poisoning seemed likely, and might offer a means of detecting fluorosis in cases where gross symptoms were not in evidence.

Blood samples were taken from heifers during their first lactation as follows: (1) prior to parturition; (2) near the peak of production; (3) mid-lactation or later; (4) and again near the end of lactation. Six lots of 3 animals each were available. Three of these were lots receiving no known source of fluorine, while the three remaining lots received approximately .02

per cent., .04 per cent. and .087 per cent. of the grain ration as fluorine fed as a mineral supplement in the form of raw rock phosphate. The rations were balanced as to protein. They contained ample energy and were in all respects adequate dairy rations.

The plasma phosphatase was determined by the method of Kay³ except that pH values were determined by means of the quinhydrone electrode rather than by the colorimetric method. In all cases the animals receiving fluorine showed a distinct rise in the plasma phosphatase values over that of the control cows. In nearly all cases the values for our control animals were within the normal range of .1000 to .2000 units per cc for mature animals. Twenty-eight determinations made upon control cows gave a range of from .1168 units to .2440 units per cc with a mean value of .1763 units. The low fluorine lot gave an average phosphatase value of .2366 units per cc. The intermediate group showed a further rise in phosphatase with an average of .2751 units per cc, while the high fluorine lot varied from .2240 units to .5312 units per cc. The mean value for this lot (12 analyses) was .3366 units per cc or practically double that of the control animals. It would seem, therefore, that in cattle suffering from fluorosis the plasma phosphatase rises in proportion to the level of fluorine intake, or nearly so. Other blood constituents, such as serum calcium, inorganic phosphorus, total phosphorus, lecithin phosphorus and chlorine, remained within the normal range. There seemed to be a tendency for the serum calcium of the blood to decrease with a correspondingly slight increase in inorganic phosphorus in the animals most severely affected.

The plasma phosphatase value is apparently an excellent index of the degree of fluorosis in cattle. Not only was there a definite gradation in the plasma phosphatase content between lots, but there was a progressive rise in plasma phosphatase in the high fluorine lot coincident with a progressive severity of the gross symptoms in the cattle. These changes are no doubt explained by the increased grain required to meet the needs of lactation and subsequent higher fluorine ingestion. Cows on the high fluorine (.087 per cent. of the grain ration) gave the following average plasma phosphatase values: at parturition 0.2787 units per cc; near the peak of production, 0.3142 units per cc; mid-lactation, 0.3537 units per cc; and at the close of the first lactation, 0.4227 units per cc. In the absence of other bone diseases the plasma phosphatase in fluorosis forms a sensitive test for the toxic effects of chronic fluorine poisoning. Similar results have also been obtained with swine and rats, although the data with these species are as yet inadequate. The rise in plasma phosphatase indicates

¹ R. Robison, *Biochem. Jour.*, 17: 286, 1923.

² H. D. Kay, *J. Biol. Chem.*, 89: 249, 1930.

³ H. D. Kay, *J. Biol. Chem.*, 89: 235, 1930.