to oxidative destruction during the filtration process. In two experiments in which parallel filtrates were made, one in the usual manner, the other with the addition of 1–500 cystein, and protecting as far as possible from the air with liquid paraffin, the latter type of filtrate in each experiment was very much more active than that made in the ordinary way.

It is therefore pretty clear that oxidation will inactivate filtrates of the Rous tumor, although the significance of this fact for a better understanding of the nature of the filterable agent is still not clear. Neill has shown that certain bacterial toxins and enzymes are subject to a reversible oxidation and reduction and it becomes of considerable importance to know whether this "virus" inactivation may be subject to a similar reactivation by means of reducing substances. Up to the present, no clear-cut evidence for this has been obtained, although there have been some indications that it may occur. Should this prove to be the case, the bearing on the interpretation of Gye's complex experiments is obvious.

It is interesting to speculate on how far this property of the filterable chicken tumor agent may be paralleled by other filterable viruses. A number of these, at any rate, exhibit the same phenomenon of rather rapid loss of infectiousness on incubation, and the question is now being studied in this laboratory.

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## VELOCITY OF CADMIUM ATOMS REGULARLY REFLECTED FROM A ROCK SALT CRYSTAL

WE have previously shown that a beam of cadmium atoms incident upon a cleavage face of a rock salt crystal is reflected so that the incident and reflected beams make equal angles with the normal to the crystal surface. At that time we suggested that this phenomenon could be interpreted in terms of the phase waves of de Broglie. The existence of a reflected beam making the same angle with the normal as does the incident beam suggests at once the possibility that we have here a situation in which the phase waves behave as X-rays do in the Bragg type of reflection.

We have now measured the velocity and velocity distribution of such reflected beams for three angles of incidence, 22.5°, 45° and 67.5°, using a rotating sectored disc velocity filter in the reflected beam. We find that within the limits of resolution of our apparatus the reflected beam is "monochromatic," *i.e.*, it contains atoms whose velocities are very nearly the same. The velocity of the atoms in the specularly reflected beam is independent of temperature of the reflecting crystal for temperatures from 200° to 500° C.

The results are given in the table:

| $\vartheta$ | Velocity observed m./sec. | Velocity calculated m./sec. |
|---|---|---|
| 22.5° | 500 | 494 |
| 45° | 530 | 530 |
| 67.5° | 600 | 605 |

The assumption of de Broglie is that there is associated with a particle of mass M and velocity V a phase wave of wave length $\lambda = \frac{h}{MV}$. We wish now to assume that this equation applies only to the three elementary particles, the photon, electron and proton, and that the wave length associated with an atom whose velocity is V is $\frac{h}{MV}$ where M is the mass of a proton, and not the mass of the atom.

That is to say, we assume that the fundamental periodicity associated with a proton does not change when it combines with other protons to form an atom. We will further assume, following Eckart,[1] that the velocity of phase waves is not the same in a crystal as in free space, so that the form of Bragg law to be used is that used by Davisson and Germer[2] in their work on reflection of electrons by crystals of nickel. That is

$$n\lambda = \frac{nh}{MV} = 2d\ (\mu^2 - \mathrm{Sin}^2\vartheta)^{\frac{1}{2}}$$

If n were greater than one we would find two or more velocities in the reflected beam. With n equal one we have but one arbitrary constant, $\mu$, the refractive index for phase waves. Using the velocity of the atoms reflected at 45° we find $\mu = 1.50$ and putting this value in the equation we get for the velocities to be expected at 22.5° and 67.5° values of 494 and 605 meters per second, as compared with observed values of five hundred and six hundred meters per second, respectively.

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## STAINING REACTIONS OF FERN GAMETES

THE work of Naylor[1] demonstrated that the retention of stains by fixed and sectioned plant tissues varies with the reaction of the liquids used in washing the slides. The dead cytoplasm of such plants as Naylor used (hyacinth, lupine, etc.), retains basic dyes when washed in buffer solutions alkaline to pH 4.6, but loses them when washed in more acid solu-

[1] Eckart, Proc. Nat. Acad. Sci., 13, 460, 1927.

[2] Davisson and Germer, Proc. Nat. Acad. Sci., 14, 317, 1928.

[1] *Amer. Jour. Bot.*, 13: 265–275, 1926.

tions; acid dyes are retained in solutions more acid than pH 5.0, but lost in solutions alkaline to this point. The material behaves in this respect somewhat like a pure protein, with the difference that its isoelectric "point" (as measured by the retention of dyes) is a range rather than a point; which is taken as indicating that the cytoplasm is a mixture of proteins. With this limitation in mind, we can say that its isoelectric point is from pH 4.6 to pH 5.0. Naylor found that the different parts of the cell had different isoelectric points, that of the chromatin of the resting nucleus being more alkaline (about ph 5.2) than that of the cytoplasm.

Methods similar to those of Naylor have been applied to fern antherozoids (microgametes), with interesting results. The prothallia of *Pteris longifolia* L. were grown in culture. Abundant antherozoids were obtained by mounting prothallia in a drop of distilled water on a slide. They were fixed by inverting the slide over 1 per cent. osmic acid for thirty seconds. After drying, the slides were immersed in stains (1 per cent. aqueous solutions) and afterwards washed in buffer solutions composed of $\frac{M}{200}$ potassium acid phthalate and sodium hydroxide. The buffer solutions varied in hydrogen-ion concentration by intervals or 0.2 pH or 0.3 pH from pH 4.1 to pH 6.0. Since the sodium content has been found to affect the staining, it was held constant by adding suitable amounts of sodium chloride.

The nucelus of the antherozoid retained the acid dyes and lost the basic dyes in buffer solutions acid to pH 4.5 or thereabouts, and detained the basic dyes and lost the acid dyes in more alkaline solutions. Its isoelectric point, as indicated by this method, is therefore in the neighborhood of pH 4.5. That of the cytoplasm, as determined in the same slides, is near pH 5.0. Good differential staining was obtained by the following procedure. The slide was immersed in safranin, (a basic dye) for twenty seconds, then destained in a buffer solution of pH 4.1. The cytoplasm lost the stain most readily, since its isoelectric point is more alkaline; the nucleus remained red for some time. Before the nucleus lost its red color the slide was stained in methyl blue (an acid dye for one minute, and then washed in a buffer solution of pH 5.2. This reaction caused the nucleus to lose the blue dye, while the cytoplasm retained it. The slide was then allowed to dry, and sealed in the usual manner. The cytoplasm was stained blue, the nucleus red. Steil's method[2] for differential staining of fern antherozoids also involves the use of an acid and a basic dye (acid fuchsin and safranin), though it includes no control of the reaction of the liquids used in washing. His method also was used by the present writers. The cytoplasm was stained faintly bluish-pink by the acid fuchsin, the nucleus bright red by the safranin.

The chief interest in these results lies in the fact that the nucleus has evidently a more acid isoelectric point than the cytoplasm, and hence behaves like an acid at reactions near pH 5.0, while at the same reaction the cytoplasm may act like a neutral or even alkaline substance. The cytoplasm of the vegetative cells studied by Naylor approximates the cytoplasm of the fern antherozoid in its isoelectric point; but the chromatin of the former cells has a more alkaline isoelectric point (though perhaps somewhat less so during mitosis). It remains, of course, to study by the same methods the vegetative cells and the megagametes (eggs) of the fern. If it is true that the antherozoid nucleus (and the antherozoid is largely nucleus) is more acid at ordinary reactions than its cytoplasm and more acid than either nuclei or cytoplasm of other cells, it may be possible to correlate this property with the physiology of fertilization. Well-known experiments[3] on both plants and animals have shown that the function of the microgamete in initiating development of the megagamete may be simulated by the addition of an organic acid.

The structure of the antherozoid observed in these experiments was that usually described. The nucleus, long, narrow and spirally coiled, occupies most of the cell; the cytoplasm forms a thin envelope around the nucleus and a projecting anterior portion. The blepharoplast, lying along one margin of the cytoplasm, retains the same dyes as does the cytoplasm at each reaction but in greater amounts. The numerous cilia arise from the anterior portion of this body. In many slides the nucleus seemed not homogeneous but composed of small granules; sometimes these gave it a beaded appearance. It is not known whether this appearance is normal or due to the age or death of the antherozoid. Probably the nucleus consists of densely aggregated chromatin in a condition similar to that of the microgamete nuclei of Bryophytes and some Spermatophytes during fertilization. This condition is usually regarded as being comparable to that of the chromatin in the prophases of mitosis; though in ferns, the zygote nucleus, when first formed, is in the resting condition.

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[2] *Bot. Gaz.*, 65: 562–563, 1918.

[3] Lillie, F. R., "Problems of Fertilization," Univ. of Chicago, 1919.