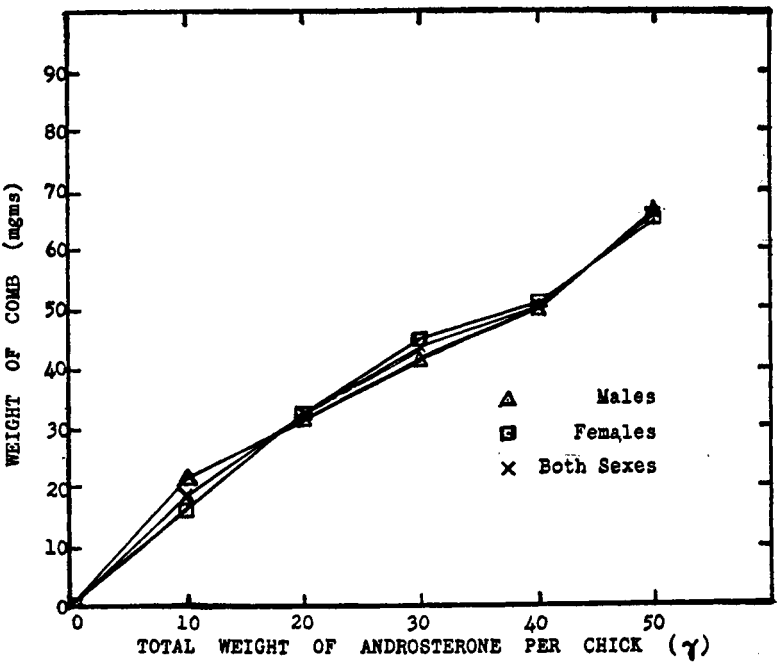

FIG. 2.

Relations between comb weight and androsterone dosage for all 5 series combined, of 582 chicks, corrected for control values.

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Penetration of Radioactive Ions, Their Accumulation by Protoplasm of Living Cells (*Nitella coronata*).

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Exploratory experiments with K*, Na*, Rb* and Br*† reveal hitherto unknown steps of the process of penetration of these ions

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† We shall refer to radioactive isotopes of elements as K*, Na*, etc., in contrast to the inactive elements, K, Na, etc.

into living cells. Studies of plant cells with K*[1, 2, 3] have already shown that it is indispensable to separate the protoplasm from the sap, and to study both of these portions separately. Animal cells lack an obvious vacuole, but experiments with erythrocytes *in vivo* show that the absorption or removal of ions from the blood as a whole takes place. The amount of ions was deduced from the emission of β particles, using a Geiger-Müller counter and the ratio of the count to the known amount of the radioactive salt used.‡ When K*Cl is injected in a *ca,* isotonic solution into a rabbit (which has K rich erythrocytes) the K* disappears from the plasma mainly by excretion or absorption, but apparently enters the erythrocytes to approximate diffusion equilibrium but without conspicuous accumulation. When Na*Cl is injected neither loss from the blood nor accumulation of Na* takes place conspicuously. It is hoped that more significant data can be obtained by experiments *in vitro.*

Earlier experiments with *Nitella* show that K* does not accumulate to any great extent (*i. e.,* attain concentrations exceeding those of the immersion fluid) in the *sap* during about the first 10 hours. But during this period we can find pronounced accumulation of ions in the *protoplasm,* the concentrations being often 10 or 25 times as high in the protoplasm as in the immersion fluid. In this accumulation we can distinguish 3 or 4 phases, shown by K*: (1) a rapid intake of K*, usually complete within 1 or 2 hours, which may be an exchange of K* from the solution for K from the cell; (2) loss of K* from the protoplasm continuing for 2 to 4 hours, and possibly due to the slower entrance of other cations, replacing K* and K. This exchange is relatively slow because these ions have other properties (*e. g.,* Na) or a lower concentration (*e. g.,* H) in exchange for the K* already taken in. (3) Steady intake of K* possibly due to exchange of K* from the medium for metabolic products, *viz.,* weak electrolyte cations.

In one experiment 3 lots of cells were first exposed to 0.01 M KCl for 4 days, while one lot was not so treated. Then the control cells were exposed to 0.01 M K*Cl while the treated lots were exposed to 0.0033, 0.01 and 0.03 M K*Cl respectively. During Phase 1 the cells took in K* into the protoplasm in proportion to the K content of the cells as shown by the difference between the control

[1] Brooks, S. C., *Trans. Faraday Soc.*, 1937, **33**, 1002.

[2] Brooks, S. C., *Am. Nat.*, 1938, **72**, 124.

[3] Brooks, S. C., *J. Cell. Comp. Physiol.*, 1938, **11**, 247.

‡ Experimental details are given in reference 3 in essentially the form used in the present work.

cells and similar cells which presumably took in K during the previous immersion in KCl. Furthermore, the amount of K* taken in by cells first treated and then exposed to 0.0033, 0.01 and 0.03 M K*Cl was at least approximately proportional to those concentrations. In contrast with this, during Phase 3, starting at different initial levels of concentration, there was no difference in the rate of intake. Penetration was independent of the external concentration of K*Cl and was dependent on forces put into play by the cell, presumably metabolic in nature.

A still later phase, with loss of K* from the protoplasm, can be distinguished as Phase 4. It occurred after 12 to 18 hours. This may result from injury, and was usually accompanied by increase of K* in the sap at this stage.

Similar experiments with *Nitella* previously immersed in NaCl instead of KCl and then in Na*Cl, showed that Phase I led to intake of Na* proportional to the external concentration of Na*Cl, as in the case of K*. But there is little difference between cells previously exposed to NaCl and not so exposed. We may conclude that Na is not taken in rapidly, although Na* can be rapidly exchanged for K or Na from the cell. After Phase 2 brought the concentration of Na* to a lower level, Phase 3 revealed almost no intake, confirming the low rate of entrance of Na.

Comparison of the rate of Na*, K* and Rb* during Phase 3, which may reflect the permeability of the protoplasm to different ions, yielded values of 0.001, 0.01 and 0.005 M/hour respectively. The data for Rb* were obtained under different conditions, but if we assume the validity of all the points, we may conclude that the penetrability of K>Rb>Na.

For Br* we find that under comparable conditions, (1) there is no Phase 1 exchange, possibly because there is no Br in the normal cell; (2) Br* may penetrate from a distilled water solution of KBr* (pH 6.6) at a rate of 0.006 M/hr, while K*Cl from a distilled water solution (pH 6.6) penetrates at a rate of 0.015 M/hr.

We have also tried single experiments on the effects of pH[1,3], of various compounds which may produce considerable increase of intake of K* during Phase 1, so that they cause accumulation in the protoplasm, up to about 20 times that in the external concentration, and of CN^- 5×10^{-4} M, which increases the K* concentration in the protoplasm as observed after 5 hours' exposure to K*Cl, while less K* gets into the sap at that time. The later stages show differences according to the pH, using 1×10^{-4} M buffers: phosphates at 5.75 and 6.68, and borate at pH 8.0.