

external jugular veins of the recipient and the central end of a similar vein in the donor, omitting the opening of the vein in the left side of the recipient's neck. This prevents the early termination of the experiment by the exsanguination of the donor.¹

By this procedure, the arterial supply of the recipient's head and neck is maintained through the right vertebral and left common carotid arteries, both of which are branches of the brachiocephalic artery. The venous outflow is maintained by the unobstructed passage of the blood into the superior vena cava from which it escapes through the external jugular vein on the right or left side as the case may be.

Injections may be made into the cerebral circulation of the recipient through a hypodermic needle inserted in the carotid artery in the region of anastomosis, or through a canula placed in the central end (clamped) of the superior thyroid artery. It is also evident that the solution may be given intravenously in the donor, but under such circumstances the influence of changes in the arterial blood pressure of the donor must be considered.

By this method of isolation, physiological experimentation upon altered cerebral blood pressure is rendered feasible by (1) diminishing the venous outflow through the jugular anastomosis, (2) by constriction of the arterial anastomosis, or (3) by raising the donor's general blood pressure.

The success of this method of isolation is evidenced by the fact that an injection of horse serum into the cerebral circulation of a sensitized animal does not cause the typical fall of blood pressure which is characteristic of anaphylactic shock, while a subsequent injection into the trunk does cause such a fall.

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A reversion of the starch-dextrin reaction.

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In the reversions of enzymic and catalytic processes heretofore recorded, the synthesis has been brought about by a high concen-

¹ Before making the venous anastomosis it is essential to ascertain whether there is an efficient back flow from the superior vena cava through the central end of the recipient's right external jugular vein. To provide this it is frequently necessary to rupture the valves that are present just above the subclavian branch.

tration of the products of analysis, or by the addition of *fresh* enzyme to a solution that had reached a state of equilibrium. In the starch dextrin reversion the synthesis was attempted in an entirely different and original way, that is, by a rapid increase of temperature of a solution that is near or at the point of equilibrium in relation to substances studied. In accordance with a known law this change of temperature should move the point of equilibrium *endothermically*.

In studying this phenomenon it must be borne in mind that in the digestion of liquid boiled starch there may be going on coincidentally at least three independently but serially related processes which are dependent upon the activities of three enzymes — one, in the depolymerization of the liquid starch into dextrans (*amylase*); another, in the hydrolysis of dextrans into maltose (*dextrinase*); and another, in the hydrolysis of maltose into glucose (*glucase*). Each of these enzymes has its own optimal point of activity; each carries on its own reaction independently of the others; and in each reaction the substances entering into the reaction have their own point of equilibrium of solution independently of the other reactions. Hence it follows that each reaction, in so far as concerns the reversion phenomenon in relation to the enzyme and the concentration of the solution and the temperature, must be studied separately. Conditions may be present that are favorable to the reversion of one of the reactions but not of the others.

In most of these experiments there was used 50 c.c. of a one per cent. solution of boiled starch with 25 c.c. of a three per cent. solution of Merck's "pure pancreatin." In a few experiments ptyalin, malt diastase or Taka-diastase was substituted for pancreatin, and in some modifications were made in the concentration of the enzyme. Digestion was usually carried on at room temperature, but sometimes at 37° C. The chief procedures and results may be summarized as follows:

1. If at any time after the reaction has proceeded so far that the addition of a two per cent. Lugol's solution yields a *violet* reaction (even though there be but the merest trace of coloration) the preparation be heated quickly to the optimal point of saccharification (approximately 60° to 65°) the composition of the solution is so altered that the addition of iodine now yields a *blue* reaction,

which is accepted as being characteristic of starch. (Claude Bernard found a form of glycogen in paralyzed muscles which gave a blue reaction with iodine.) The blue coloration is proportional to but more intense than the corresponding violet previously obtained, so that even when the violet coloration was scarcely discernible a good blue reaction was observed. The optimal temperature of the starch-dextrin reaction is distinctly higher than the temperature at which the reversion occurs, but the exact point has not been determined. The occurrence of the reversion at the optimal temperature of saccharification is doubtless merely a coincidence.

2. When digestion has proceeded to the point at which there is not a color reaction with iodine, in other words to the achroödextrin-sugar stage, heating the preparation as above did not cause either a starch-dextrin or an erythro-dextrin-achroödextrin reversion, as was shown by the continued absence of any color reaction with iodine.

3. By testing the preparations before and after heating with the usual copper-reducing tests, and with the polariscope, both dextrin-maltose and maltose-glucose reversions were occasionally detected, but the results were very inconstant and generally not absolutely conclusive. The absence of a constant occurrence of these reversions is doubtless owing to a failure to obtain the exact concentration of solution in relation to temperature.

4. The starch-dextrin reversion is not permanent unless the preparation has been heated to the temperature at which the enzyme is destroyed. Upon cooling a preparation that has been heated to only a lower level a reconversion of the starch into dextrin occurs, with of course a loss of the blue reaction and a return of the violet reaction with iodine.

5. The starch-dextrin reversion is not enzymic, but dynamic, and, therefore, the mechanism is quite different from that of the reversions recorded by previous observers. Formaldehyde in even small amounts is, as is well known, highly destructive to amylase, and when added to the starch-dextrin solution absolutely prevents the reversion. This might be taken as showing that the absence of reversion is due to the destruction of the enzyme, but this is negated by the fact that the addition of strong mineral acids in

quantities sufficient to instantly destroy the enzyme does not prevent reversion. Unusual interest is attached to the starch-dextrin reversion, not only because of the phenomenon *per se*, but also because it is not enzymic, and because formaldehyde prevents it. How formaldehyde is effective is problematical.

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The rôle of alkali in the development of the sea-urchin.

By **JACQUES LOEB.**

In a former paper (1898) I have shown that the velocity of development of the eggs, is within certain limits, a function of the concentration of the hydroxylions in the surrounding solution; and I pointed out the probable connection of the action of bases with oxidations. In a later paper (1906) it was shown that at a concentration of hydroxylions below, but very close to, the point where neutral red indicates an alkaline reaction (*i. e.*, near the point of neutrality) the eggs cannot develop beyond the eight cell stage.

If we put fertilized and unfertilized eggs of *Purpuratus* into seawater to which a drop of neutral red has been added, at first, the fertilized and unfertilized eggs take the stain equally well. If we later transfer the eggs into seawater which is free from neutral red, the fertilized eggs gradually take all the stain while the unfertilized eggs become in the same measure decolorized. The explanation for this phenomenon lies in the fact that in the fertilized egg the neutral red enters into a chemical combination by which it becomes undiffusible; while in the unfertilized egg the neutral red is only held in solution. Since neutral red is a base it is to be presumed that the body in the egg with which it combines is an acid.

This suggested the possibility that the above mentioned acceleration of the development of the egg by other bases, *e. g.*, sodium or potassium hydroxide, might be due to a combination of these bases with the same acid with which neutral red combines in the fertilized egg. If this assumption were correct it should be expected that the addition of two or three drops of a 1/100 gram-molecular solution of neutral red to a neutral van't Hoff solution (in which the fertilized eggs cannot develop) should cause the eggs to develop into swimming larvæ. The experiment was tried and it was found that neutral red has indeed such an effect.