

processes. Even a solution of 1½ per cent. of formalin in 95 per cent. alcohol may exert these effects on human anterior pituitary. Solutions of formalin in H<sub>2</sub>O or 0.9 per cent. NaCl solution, especially after addition of a small amount of alkali, act therefore in principle in about the same way on human and on cattle anterior pituitaries and make both similar in their action to the anterior pituitary of the guinea pig.

(4) If human or cattle anterior pituitaries are placed for several days in H<sub>2</sub>O, 0.9 per cent. NaCl or glycerine, effects 3 and 4 (types I and II of luteinization) are accentuated, while process 1 (follicular growth and maturation) is weakened. Anterior pituitaries thus treated also stimulate the thyroid gland. After an immersion lasting 1 day in these solutions, the maturation effect may still be present.

(5) Likewise, 50 per cent. or 95 per cent. alcohol and ether, acting for 3 to 7 days on cattle or human anterior pituitaries, cause a predominance of the I and II luteinization processes in the effects exerted by these glands; also the thyroid gland is stimulated. In some cases growth-maturation processes may be combined with luteinization processes and occasionally even an injurious action on follicles may be noticeable. Human anterior pituitary kept for four weeks in 95 per cent. alcohol may still produce strong luteinization effects of type II. If the anterior pituitary is exposed to the action of 95 per cent. alcohol for only 1 day, growth-maturation processes may be marked. There was observed usually an inverse relation between the hypertrophy of the thyroid gland and the intensity of the growth-maturation process in the ovary.

(6) Of the other substances tested we shall mention only 50 per cent. alcohol saturated with Na<sub>2</sub>SO<sub>4</sub>; the effects of this solution on human anterior pituitary may be similar to those which weak solutions of formalin exert, although they are not so pronounced in the former solutions as in the latter.

(7) While thus, as a rule, there was a definite correlation between thyroid hypertrophy and luteinization processes, especially of type II or with injury (atresia) of the ovarian follicles, and an inverse correlation between thyroid hypertrophy and growth-maturation of follicles, it was possible, as we have seen, to separate experimentally the thyroid-stimulating hormone from the substance producing atresia of follicles; the former was still active in cattle anterior pituitaries which no longer injured the ovarian follicles. In some cases it was also possible to disassociate experimentally the thyroid-stimulating hormone from the hormone or hormones causing luteinization processes types I and especially II. Furthermore, the previously untreated anterior pituitary gland from a woman who had died during the period of lactation produced only very marked follicular growth-maturation effects but no

luteinization processes and still it exerted a strongly stimulating action on the thyroid gland.

We may therefore conclude that while usually the thyroid-stimulating hormone is in some way associated with the luteinizing hormone and with the follicle-injuring substance—a relation to which we called attention several years ago<sup>2</sup>—this connection is not a necessary one, an experimental dissociation between these various effects of the anterior pituitary being possible and occurring also under natural conditions in certain cases. The thyroid-stimulating hormone is therefore in all probability not identical with the luteinizing or follicle-destroying substance.

(8) These experiments have shown that it is possible to transform the action of the anterior pituitary of one species into that of another species and that the presence of one substance in the anterior pituitary may cover up the presence of other substances which become manifest after experimental removal of the first substance.

(9) The data obtained may be interpreted by assuming that the effect of these various hormones depends upon the presence of certain amino-acids, which form part of one or several polypeptid or protein molecules. The amino-acids, responsible for the stimulation of the thyroid and for the luteinization processes, especially of type II, would be very similar in constitution and, therefore, would be affected by formalin in a similar manner. On the other hand the chemical group responsible for growth-maturation processes would be more resistant to the action of formalin. We consider this interpretation merely as a suggestion, which may perhaps be serviceable in explaining the results of these investigations.

LEO LOEB  
W. C. ANDERSON  
JOHN SAXTON  
S. J. HAYWARD  
A. A. KIPPEN

#### TRAVERTINE DEPOSITING WATERS NEAR LEXINGTON, VIRGINIA

THE travertine depositing waters of the lower, cascading part of Wilson Falls Creek, 18 miles northeast of Lexington, Virginia, are supersaturated with Ca(HCO)<sub>2</sub> throughout the year, the excess ranging from about 68 to 76 parts of CaCO<sub>3</sub> per million. Not counting high-water periods, the largest excess appears in winter. These conclusions are based on monthly analyses of the creek and of a feeder spring over a span of one year. The creek was sampled at 4 stations. The first and last stations are about a mile

<sup>2</sup> Leo Loeb and R. B. Bassett, *Proc. Soc. Exp. Biol. and Med.*, 27: 490, 1930; Leo Loeb, *Endocrinology*, 16: 129, 1932; *Proc. Soc. Exp. Biol. and Med.*, 29: 642, 1932.

apart, and their vertical interval is about 300 feet. The drop between the second and third stations, at the top and bottom of Cypress Falls, respectively, is 130 feet.

The annual range of the pH, temperature, free  $\text{CO}_2$  and of some other constituents of spring and stream are given in Table 1. Column 5 shows the  $\text{CaCO}_3$

A solution with 200 parts of  $\text{CaCO}_3$  per million and only a few parts of free  $\text{CO}_2$  was adjusted, that is, reduced to saturation, by vigorous aeration in the presence of calcite in 28 hours. When aerated without calcite, the same solution still had an excess of 15 parts per million after 19 days of aeration. Aerated solutions when placed in stoppered flasks with calcite

TABLE I

|                         | 1       | 2                  | 3           | 4               | 5                                        | 6               | 7             | 8  |
|-------------------------|---------|--------------------|-------------|-----------------|------------------------------------------|-----------------|---------------|----|
|                         | pH      | Free $\text{CO}_2$ | Temperature | $\text{CaCO}_3$ | $\text{CaCO}_3$<br>saturated<br>solution | $\text{MgCO}_3$ | $\text{SO}_4$ | Cl |
| Feeder spring . . . . . | 7.1     | 19                 | 11°–15° C.  | 165–190         | 65–68                                    | 41–52           | 1 > 5         | tr |
| Wilson Falls Creek ..   | 7.8–8.4 | 0–3                | 6.5°–25° C. | 123–156         | 55–80                                    | 37–86           | 1 > 5         | tr |

content assigned by theory to saturated solutions having the temperature range indicated in column 3 and a partial  $\text{CO}_2$  pressure of .0003, this partial pressure being about the same as the nearly constant  $\text{CO}_2$  pressure of the air. All compounds are given in parts per million.

At temperatures below 8° C., the  $\text{CaCO}_3$  content was about the same at all stations, indicating no deposition. Likewise, pH and free  $\text{CO}_2$  content showed the least variation at the lowest temperatures. The summer record shows the greatest differences between stations. The lowest pH and the highest free  $\text{CO}_2$  and  $\text{CaCO}_3$  came from the highest station.  $\text{CaCO}_3$  was lowest at the last station, while pH was highest at the base of Cypress Falls as a rule. The latter also recorded  $\text{CO}_2$  lacking or very low. The greatest difference in the  $\text{CaCO}_3$  of the first and last station was about 25 parts per million.

The creek waters are highly supersaturated  $\text{Ca}(\text{HCO})_2$  solutions because the inflowing spring waters are even more supersaturated and because the adjustment of such solutions to the partial  $\text{CO}_2$  pressure of the atmosphere is amazingly slow. Note the low pH, and the high free  $\text{CO}_2$  and  $\text{CaCO}_3$  content of the spring in the preceding tabulation. The supersaturation of the springs is believed to result from the high partial  $\text{CO}_2$  pressure of soil and subsoil gases, reported as commonly ranging from 30 to 270 times greater than that of the air. That this high  $\text{CO}_2$  content of subsurface gases is in some direct relation to the humus cover is shown by springs in unbroken forest having the highest concentration of  $\text{Ca}(\text{HCO})_2$  in the Lexington area.

The marked deposition of calcite by the summer waters is due mainly to rise in temperature. The summer waters are warmer than the spring waters, and this hastens adjustment. Adjustment is also hastened by aeration and close contact of the water with calcite, but these factors are in effect at all times. Contact with calcite is most effective at low-water stages.

for 20 to 48 hours lost  $\text{CaCO}_3$  when the concentration was more than 80 parts, initially. There was no air space above the solutions. The greatest loss in the flasks was 40 parts per million and came after the first and second hour of aeration.  $\text{CO}_2$  was liberated in the flasks, but the amount liberated was much smaller than it should have been if the  $\text{CaCO}_3$  lost from the solution had been in the bicarbonate form. The carbonate radicle seemed to be absent. The facts suggest that much of the  $\text{CaCO}_3$  which appeared to be in solution was really in a crystalline colloidal state, too fine grained to be retained by the filter. Check samples of the aerated solutions when placed in stoppered flasks without calcite did not lose  $\text{CaCO}_3$ . Calcite also hastened the adjustment of supersaturated solutions when standing quietly in air. With calcite the rate was nearly doubled.

The floral film of the cascades, mostly algal at present, can have but little effect on the adjustment of the relatively thick sheet of water sweeping over it. Prior to cultivation and the present entrenchment of the streams, plants may have been more effective. Then the waters were spread out in thin films, trickles and drips. Calcite is not deposited upon the growing top foliage, but upon the basal part of the plant, and even more upon the stifled foliage underneath. Travertine grows from the base up by addition of calcite to calcite, which seems to be a result of the catalytic action of calcite on unstable  $\text{CaCO}_3$  solutions. By this addition of calcite to calcite, the rootless plants of the cascades become rooted and live more securely. In turn, the plants form both a framework and a protective cover for the growing travertine, a unique example of mutual aid between the organic and the crystalline.

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EDWARD STEIDTMANN

VIRGINIA MILITARY INSTITUTE