taneously with progesterone. A representative series is presented in Table 1. The data indicate further

TABLE 2

AVIDIN TITRE OF OVIDUCTS OF STILBESTROL PRETREATED (0.5 MG. DAILY FOR 6–8 DAYS) CHICKS AUTOPSIED 2 TO 16 HOURS FOLLOWING SUBCUTANEOUS INJECTION OF PROGESTERONE (+ STILBESTROL). FIGURES IN PARENTHESES INDICATE RANGE OF TITRES

Injection to autopsy	Secondary injection			
	Progesterone 0.4 mg Stilbestrol 1.0 mg		Progesterone 1.6 mg Stilbestrol 4.0 mg	
hours	*oviducts*	*avidin, units*	*oviducts*	*avidin, units*
2	3	0.13 (0–0.25)	3	0.32 (0.20–0.50)
3	3	0.44 (0.33–0.50)	3	0.24 (0.20–0.33)
4	7	0.71 (0.20–1.60)	3	0.26 (0.20–0.33)
8	5	0.55 (0–1.00)	3	1.18 (0.33–2.00)
16	5	0.40 (0.33–0.50)	3	1.47 (1.00–2.00)

that the avidin titre increases with increasing progesterone over at least a 16-fold range, whether stilbestrol is administered simultaneously or not. Moreover, similar reciprocal quantitative relations are observed for desoxycorticosterone as for progesterone. Thus there is no evidence of the decisive antagonism between oestrogen and progesterone which is observed in the progestational response of the mammalian uterus.

These quantitative and qualitative features of the endocrine control of avidin formation lend additional support to the possibility that avidin may play a role in the physiology of reproduction.

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ON THE VITAMIN B_C CONJUGATE IN YEAST

THE isolation of crystalline vitamin B_C from liver and its identity with an *L. casei* growth factor has been reported.[1] We have found that yeast and certain yeast extracts are highly active in vitamin B_C activity as measured in the anemic chick but they have little potency in stimulating the growth of *L. casei*. Only about 2 to 5 per cent. of the chick antianemia activity can be accounted for in terms of microbiological growth effect on either *L. casei* or *S. lactis*. The methods used for isolating vitamin B_C from liver failed when applied to yeast. Using the chick curative assay procedure[2] and later a preventive assay procedure[3] we have concentrated the chick antianemia factor in yeast. It is seemingly non-protein in nature, since it is dialyzable through Cellophane No. 300, and is not precipitated by heat in acid solution, by saturated ammonium sulfate at pH levels between 3 and 7 nor by trichloracetic acid.

Concentrates of the chick antianemia factor which are essentially inert in stimulating the growth of *L. casei* become highly active in microbiological growth effect following enzymatic digestion. Procedures developed earlier for the isolation of vitamin B_C from liver when applied to such digests yielded a pure crystalline compound which had the same growth-stimulating activity on *L. casei* and *S. lactis* as vitamin B_C from liver. It also had a comparable effect on the blood picture and growth of the chick. The products from the two sources have the same color, crystalline appearance and solubilities. They behave similarly on heating, slowly discoloring and charring from about 250° C. without melting. The compounds from liver and yeast were found to be crystallographically identical[4] and to have identical ultraviolet absorption spectra.[5] They analyzed as follows: Yeast compound, C 52.44, 52.51; H 4.37, 4.41; N 20.3, 20.2; liver compound, C 52.44, 52.46; H 4.28, 4.49; N 19.8, 19.6.[6] We conclude that the compound isolated from yeast is identical with vitamin B_C from liver.

Stokstad[7] on the other hand has reported the preparation of an *L. casei* factor from liver and a different *L. casei* factor from yeast. He believes the one from liver to be identical with vitamin B_C. More recently Hutchings *et al.*[8] have presented evidence for the existence of at least three *L. casei* factors or "folic acids." The source of the third one is not stated.

The fact that our crystalline product from yeast, a plant source, is identical with crystalline vitamin B_C from liver increases the probability that the "folic acid" concentrates prepared from spinach by Mitchell, Snell and Williams[9] contained variable amounts of vitamin B_C.

Our results demonstrate that the chick antianemia activity in yeast extract is due to the presence of vitamin B_C held almost entirely in the form of a rela-

[1] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, SCIENCE, 97: 404, 1943.

[2] B. L. O'Dell and A. G. Hogan, *Jour. Biol. Chem.*, 149: 323, 1943.

[3] C. J. Campbell, R. A. Brown and A. D. Emmett, *Jour. Biol. Chem.*, 152: 483, 1944.

[4] Observations by Professor C. B. Slawson, of the University of Michigan.

[5] E. S. Bloom, J. M. Vandenbelt, S. B. Binkley, B. L. O'Dell and J. J. Pfiffner. In press.

[6] Analytical results reported previously (SCIENCE, 97: 404, 1943) were obtained on a sample since found to be incompletely dried.

[7] E. L. R. Stokstad, *Jour. Biol. Chem.*, 149: 573, 1943.

[8] B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, J. J. Oleson and L. W. McElroy, Abst. of 107th meeting, Am. Chem. Soc., Cleveland, Ohio, April 3–7, p. 1A (1944).

[9] H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941; 66: 267, 271, 274, 1944.

tively simple conjugate. It is common knowledge that many of the B vitamins occur in a "bound" form, that is, bound to macro-molecular substances, but the occurrence of a simple non-protein conjugate of vitamin B_C has not been previously recognized. It seemed desirable to call attention to these results at this time since they bear on the interpretation of nutritional data involving growth of the chick, especially with respect to the chemical identification of other chick factors, such as Factor U,[10] the alcohol precipitate factor[11] and vitamins B_{10} and B_{11}.[12] Any one or all four of these factors may be identical with vitamin B_C conjugate.[13] Work is in progress on the chemical nature of the non-vitamin B_C portion of the conjugate molecule.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A FIVE-MINUTE METHOD FOR STAINING FECAL SMEARS

USING the following technique, it is possible to prepare permanent hematoxylin stained slides of fecal smears in five minutes or less.

Solution I (mordant-fixative)
formalin, 10 per cent. by volume ..... 3 parts
glacial acetic acid ..... 1 part
ferric ammonium sulfate ..... 3 per cent.
(3 grams of the iron alum per 100 cc formol-acid mix)
Solution II (stain)
0.5 per cent. hematoxylin (aqueous)
Solution III (dehydrating and clearing)
dioxane
Solution IV (dehydrating and clearing)
dioxane
Solution V (clearing)
dioxane and toluol, half and half.

PROCEDURE

(1) With a brush, make a very thin smear of the fecal material to be examined.

(2) Cover smear immediately with a few drops of solution I. Pass slide through a bunsen or alcohol flame a few times or until the fixative begins to steam. Be ready to add more solution to the slide if necessary to prevent drying. Quickly pour off the mordant-fixative.

(3) Immediately add several drops of solution II. Again pass the slide through the flame one or two times, tilting it back and forth. Be sure that enough stain is put on to keep the smear covered. The fecal material should become a dark purple color in three or four seconds.

(4) Place the slide at once into a coplin jar of water and wash under running tap water for one minute.

(5) Lay the slide on blotting paper and remove excess water on the smear side with filter paper or cleansing tissue. Quickly transfer the slide to solution III.

(6) Put the slide into the second jar of dioxane for at least one minute.

(7) Transfer the slide to solution V for a minimum of thirty seconds.

(8) Mount in clarite.

COMMENTS

The results obtained are much superior to those from the iodine technique for rapid fecal examination and compare favorably with the usual speed-up process using warm solutions, which takes about an hour and a half. The material is not as satisfactory as that prepared by the standard longer methods for critical cytological work. Even so, this technique is well adapted for a rapid survey of intestinal protozoa of animals or of man. Leaving the stain on for an extra second will overstain the cells but will make cilia, undulating membranes and flagella stand out clearly. Careful staining will adequately show all chromatin particles. The length of time of staining depends on the size of the flame used and the temperature of the slide. In some cases, the slide is hot enough after the mordant-fixative treatment to require no additional heating with the stain. Care must be taken, however, not to overstain the smear. If it does become overstained, it can be rapidly destained by rinsing in water and then adding a few drops of cold solution I. Stop the destaining process and blue the smear by washing in tap water. Dehydration and clearing are aided by slight agitation of the slide while

[10] E. L. R. Stokstad and P. D. V. Manning, *Jour. Biol. Chem.*, 125: 687, 1938; E. L. R. Stokstad, P. D. V. Manning and R. E. Rogers, *Jour. Biol. Chem.*, 132: 463, 1940.

[11] A. E. Schumacher, C. F. Heuser and L. C. Norris, *Jour. Biol. Chem.*, 135: 313, 1940.

[12] G. M. Briggs, Jr., T. D. Luckey, C. A. Elvehjem and E. B. Hart, *Jour. Biol. Chem.*, 148: 163, 1943; Abst. of 107th meeting, Am. Chem. Soc., Cleveland, Ohio, April 3–7, p. 15B (1944).

[13] We have demonstrated the occurrence of vitamin B_c conjugate in certain liver extracts.