tedly impractical without cooperation the method when properly employed allows recovery of 60 per cent. of the aerosolized penicillin in the urine within twelve hours, comparing favorably with an average recovery of 60 per cent. after intravenous injection.[6]

VERNON BRYSON
BIOLOGICAL LABORATORY,
COLD SPRING HARBOR, N. Y.

EVA SANSOME
CARNEGIE INSTITUTION OF WASHINGTON,
COLD SPRING HARBOR

SIDNEY LASKIN
BIOLOGICAL LABORATORY,
COLD SPRING HARBOR

ENDOCRINOLOGICAL ASPECTS OF AVIDIN FORMATION IN THE AVIAN OVIDUCT

WE have previously reported that avidin, the antibiotin factor found in egg-white, is formed normally in the oviduct of the hen and that avidin formation may be induced experimentally by the administration of progesterone to immature birds pretreated with stilbestrol.[1, 2] In the present report we wish to present data concerning (1) the ability of steroids other than progesterone to induce avidin production in previously oestrogenized chicks and (2) the quantitative aspects of the oestrogen-progesterone relationship in avidin formation.

Experimental methods, definition of the avidin unit and control data on untreated and stilbestrol treated birds have been previously described.[2] In our earlier studies we have employed chicks approximately six weeks old. We have subsequently found that the day-old chick responds equally well to the same treatment, indicating that no post-hatching reproductive development other than that occurring during the six-day course of stilbestrol conditioning is required for experimental avidin induction. In the experiments reported here we have employed chicks 42 days old with the exception of the testosterone series in which day-old chicks were used.

Both desoxycorticosterone acetate and testosterone propionate induce avidin formation, desoxycorticosterone approximating progesterone in effectiveness (Table 1). Testosterone propionate is effective at a somewhat higher daily dose (3.2 mgms), but its minimal effective dose was not determined.

This lack of specificity in avidin response to the several steroids tested is in keeping with their recognized interchangeability in such other endocrinological reactions as the maintenance of life in adrenalectomized animals and the precipitation of endometrial bleeding in the monkey.[3, 4]

The comparable effectiveness of progesterone and desoxycorticosterone also raises the question of the gonadal or extragonadal origin and of the chemical identity of the steroid normally causing avidin formation in the laying hen.

Table 2 summarizes our data on the latent period required for the appearance of avidin following subcutaneous administration of progesterone with stilbestrol. By the end of two hours avidin is readily demonstrable in the oviduct and relatively high titres are reached within 4 to 8 hours. The latent period for progesterone induction of sexual receptivity in the guinea pig is from 3 to 9 hours, an interval quite comparable with that observed for avidin formation in the chick oviduct.[5]

Since the progestational reaction in mammalian endometria is facilitated by small supplementary dosages of oestrogen but is completely obliterated by relatively large dosages, it seemed desirable to determine the effect of increased oestrogen levels upon the avidin response.[6] The avidin titre is materially elevated when increased oestrogen is administered simul-

TABLE 1

AVIDIN TITRE OF OVIDUCTS OF 6-WEEK-OLD CHICKS, PRETREATED FOR 6 DAYS WITH 0.5 MG. STILBESTROL DAILY; SECONDARY INJECTIONS FOR 2 DAYS THEREAFTER. AUTOPSY CA. 24 HOURS LATER. ALL INJECTIONS SUBCUTANEOUSLY

Secondary injections		Oviducts tested	Avidin titres	
Stilbestrol	DOCA*		Average	Range
mg. daily	*mg. daily*	*No.*	*Units*	*Units*
None	0.05	3	0	.....
"	0.20	4	0.13	0–0.25
"	0.80	4	0.48	0.33–0.60
"	3.20	7	0.56	0.50–0.60
0.5	0.80	4	0.46	0.33–0.50
"	3.20	4	1.89	1.40–2.50
5.0	0.80	4	1.95	2.50–3.30
"	3.20	3	1.28	0.33–2.50
	Progesterone *mg. daily*			
None	0.05	2	0	.....
"	0.20	3	0.17	0–0.30
"	0.80	7	0.42	0.12–0.50
"	3.20	4	1.20	0.60–1.66
0.5	0.05	4	0	.....
"	0.20	4	0.21	0–0.30
"	0.80	8	2.63	1.66–5.00
"	3.20	2	4.40	3.70–5.00
5.0	0.80	4	1.78	1.20–2.50
5.0	3.20	4	3.30	all 3.30
	Testosterone Propionate *mg. daily*			
None	3.20	3+	2.30	1.00–3.30
"	6.40	3+	0.70	0.50–1.00
"	12.80	3+	1.30	1.00–1.60

* DOCA = Desoxycorticosterone acetate.
+ = day-old chicks.

[6] C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Investigation*, 22: 425, 1943.

[1] R. M. Fraps, R. Hertz and W. H. Sebrell, *Proc. Soc. Exp. Biol. and Med.*, 52: 140, 1943.

[2] R. Hertz, R. M. Fraps and W. H. Sebrell, *Proc. Soc. Exp. Biol. and Med.*, 52: 142, 1943.

[3] R. Gaunt, W. O. Nelson and E. Loomis, *Proc. Soc. Exp. Biol. and Med.*, 39: 319, 1938.

[4] F. L. Hisaw, *Endocrinology*, 33: 39, 1943.

[5] E. W. Dempsey, R. Hertz and W. C. Young, *Am. Jour. Physiol.*, 116: 201, 1936.

[6] F. L. Hisaw and S. Leonard, *Am. Jour. Physiol.*, 92: 574, 1930.

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.

ROY HERTZ
R. M. FRAPS
W. H. SEBRELL

NATIONAL INSTITUTE OF HEALTH,
U. S. PUBLIC HEALTH SERVICE,
BETHESDA, MD.
AND
BUREAU OF ANIMAL INDUSTRY,
BELTSVILLE, MD.

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.