thesis in *Escherichia coli* extracts[9] was inhibited 50 per cent by $2 \cdot 5 \times 10^{-4} M$ tetrahydroaminopterin or N^{10}-formyltetrahydroaminopterin, one-third the concentration of the tetrahydrofolic acid present. The unreduced analogues in each case were not inhibitory under the same conditions. In contrast, tetrahydroamethopterin was no more inhibitory than amethopterin. Both compounds yielded only 20 per cent inhibition when equimolar with tetrahydrofolic acid ($7 \cdot 5 \times 10^{-4} M$).

These findings suggest that the hydrogenated analogues may be effective therapeutic agents and that they may also be useful in enzyme studies.

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Biotin in the Synthesis of Fatty Acid and Cholesterol by Mammalian Liver

Wakil, Titchener and Gibson[1] have shown that the formation of malonate from acetate and carbon dioxide is an early step in the synthesis of fatty acids in cell-free preparations of pigeon liver. They have also shown that biotin is necessary for this reaction, since it is inhibited by avidin, a specific inhibitor of biotin. By observing the effect of avidin, we have been able to show that, in cell-free preparations of rat liver, biotin is also required for the synthesis from acetate of fatty acids, but is not required for synthesis of cholesterol. It is thus possible to inhibit one biosynthetic pathway without influencing the other.

Homogenates of rat liver were prepared in ice-cold buffer by Bucher and McGarrahan's method[2], and were centrifuged for 20 min. at 2,000*g* to remove nuclei and cell debris, and then for 25 min. at 10,000*g* to remove mitochondria. The supernatant from the second centrifugation, containing the lighter subcellular particles, was transferred to flasks for incubation at 37° C. with air as the gas phase. Each flask received 2·5 ml. of supernatant, 20 μmoles of carbon-14-acetate as precursor of fatty acids and cholesterol, and the additions shown in Table 1. After incubation for 2 hr., the contents of the flasks were hydrolysed with sodium hydroxide. Fatty acids as soaps and cholesterol as digitonide were then isolated from the hydrolysate and assayed for radioactivity by methods previously described[3]. Table 1 shows the results of a typical experiment. The specific activity of the fatty acids from the flasks containing avidin without biotin was less than 10 per cent of that in the controls. Addition of biotin restored the synthesis of fatty acids almost to the control-level. Avidin had no significant effect on the specific activity of the cholesterol isolated at the end of the incubation.

Table 1. Each flask received 2·5 ml. of liver supernatant fraction and 1·0 ml. of buffer (magnesium chloride, 0·005 *M*; nicotinamide, 0·03 *M*; potassium phosphate, 0·1 *M*, *p*H 7·4) containing reduced coenzyme I, 1·0 μmole; coenzyme II, 0·5 μmole; glutathione, 30 μmoles; adenosine triphosphate, 17·5 μmoles; glucose-6-phosphate, 10 μmoles; potassium bicarbonate, 35 μmoles; potassium malonate, 25 μmoles; coenzyme A, 0·1 μmole; sodium 1-carbon-14-acetate, 20 μmoles/5 μc.

Additions	Specific activity (counts/min. at infinite thickness)	
	Fatty acids	Cholesterol
None	24,600	1,860
Biotin, 10 μgm.	32,500	1,860
Avidin (equivalent to 2 μgm. biotin)	1,910	1,690
Avidin + biotin	23,300	1,735

The avidin used in these experiments was kindly supplied by Miss H. Hellig, who prepared it by Woolley and Longsworth's method[4].

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PHYSIOLOGY

Effect of Season on the Follicle Stimulating Hormone and Luteinizing Hormone Potency of Sheep Anterior Pituitary Glands

It has been shown in the ewe that the 'total gonadotrophin' hormone content of the pituitary remains about the same during the breeding and during the non-breeding season (Warwick[1]). Kammlade *et al.*[2] noticed a rather higher total gonadotrophin hormone-level in the pituitary of the ewe during the non-breeding than during the breeding season. Lamond *et al.*[3] found no seasonal variation.

We[4] have carried out work on the change in levels of follicle stimulating hormone and luteinizing hormone (interstitial-cell stimulating hormone) activity of the pituitary glands of sheep collected at precise stages of the œstrous cycle. In this communication a comparison is made between some of these values and values obtained during the non-breeding season.

The anterior lobes of pituitary glands collected from Welsh mountain ewes kept under controlled conditions have been studied. The seasonal distribution for the collection of these glands in given in Table 1.

Table 1

Reproductive state	Time of year when samples were collected
36 hr.*	7.11.56 – 12.12.56
10 day*	20.11.56 – 14.12.56
Anœstrus	25.7.57 – 28.7.57

* From onset of œstrus.