position), a change in the opposite direction corresponds to either an inhibition of acid production, an increased production of bicarbonate or both. Further study has revealed that a major factor in the biotin effect is an increased production of bicarbonate, due to an increased utilization of lactate. This is evident from the data in the last column of Table 1, which

TABLE 1

THE EFFECT OF BIOTIN ON THE METABOLISM OF BIOTIN-DEFICIENT RAT LIVER SLICES IN THE PRESENCE OF LACTATE

Experimental conditions: 25–35 mg dry weight liver tissue in 2.2–2.4 cc Ringer-bicarbonate medium containing 0.005 M–0.02 M sodium *dl*-lactate. Gas phase: 95 per cent. O_2–5 per cent. CO_2. Temp., 38° C. Biotin concentration (when present): 1 microgram per cc of medium.

Animal No.	Diet	Liver biotin content γ/gm	Added biotin	Q_{O_2}	R.Q.	$Q_G^{O_2}$	$Q_{LA}^{O_2}$
7	B	0.8	+	−4.9	0.79	−0.15	−1.03
			−	−4.6	0.74	0.60	−0.76
42	B	0.2	+	−7.1	0.98	−0.72	−1.53
			−	−6.5	0.92	0.06	−1.23
70	B	0.1	+	−7.3	0.91	−0.78	.....
			−	−6.6	0.94	−0.31	.....
81Y	EW	0.9	+	−5.7	0.87	0.26	−1.00
			−	−5.9	0.81	0.60	−0.80
84Y	EW	0.6	+	−10.2	0.68	−2.33	.....
			−	−9.8	0.59	−1.34	.....
72Y	EW	0.3	+	−10.9	0.79	−3.53	.....
			−	−11.2	0.77	−2.32	.....

The symbols Q_{O_2}, R.Q., and $Q_G^{O_2}$ have their usual significance, $Q_{LA}^{O_2}$ refers to lactic acid values determined by chemical analysis and expressed in the same units as $Q_G^{O_2}$. Diet B contains 10% egg white and 0.1% "butter yellow." Diet EW contains 40% egg white and no "butter yellow."

gives the results of chemical analyses for lactate utilization, using the method of Barker and Summerson.[3] It is clear that added biotin results in a 25 per cent. to 35 per cent. increase in the rate of disappearance of added lactate; increases up to 50 per cent. or more have been occasionally observed. The production of bicarbonate which is measured on the manometer is due of course to the sodium remaining after the lactate has been metabolized.

When pyruvate is used as substrate, the manometric picture is quite similar to that described for lactate. Added biotin usually produces a slight rise in oxygen consumption and in the R.Q.; the effect on the Q_G values is invariably as striking as for lactate. There is no effect of added biotin when glucose is the substrate or when a non-nutrient medium is used.

About 30 minutes' incubation of the liver tissue with biotin is required before the effect of biotin becomes significant. As little biotin as 2 parts in ten million of medium have been found to have a demonstrable influence on the metabolism of the liver. Other tissues from biotin-deficient animals which have been studied include heart and brain; for neither of these could an effect of added biotin on metabolism be demonstrated, although livers from the same animals gave satisfactory results.

The analyses for the biotin content of the livers used are expressed on a dry weight basis and were run for us by Dr. Karl Dittmer and Mrs. Glenn Ellis, to whom we wish to express our appreciation. The yeast-growth method of assay was used. Normal rat livers assay from 2 to 4 micrograms per gram dry weight by this method.

Our first biotin-deficient rats came from a group which was on a biotin-low synthetic diet containing, in addition to the usual ingredients, 10 per cent. egg white, a high riboflavin content and 0.1 per cent. "butter yellow" (diet B of Table 1). The livers from these animals were essentially normal except for their low biotin content, but interpretation of our results was complicated by the presence of "butter yellow" in the diet. The experiments were therefore repeated on rats rendered biotin-deficient by a synthetic diet containing no "butter yellow" and from 30 per cent. to 40 per cent. egg white (diet EW in Table 1). No differences were noted between the two groups of animals with regard to the effect of added biotin on the respiratory metabolism of the liver slices.

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[3] S. B. Barker and W. H. Summerson, *Jour. Biol. Chem.*, 138: 535, 1941.

10-NOR-PROGESTERONE, A PHYSIOLOGICALLY ACTIVE LOWER HOMOLOG OF PROGESTERONE

ESTROGENIC activity is to a great extent independent of specific chemical structures and configurations. Syntheses have yielded a number of active estrogens which are structurally only slightly, if at all, related to the naturally occurring hormones. No comparable synthetic compounds exist in the field of androgenic, progestational and adrenal-cortical hormones. It appears that progestational and adrenal-cortical activities depend on fairly specific chemical structures and configurations.

The question arises, at what stage simplifications in the structures of the naturally occurring hormones are accompanied by the loss of physiological activity. With this problem in mind 10-nor-progesterone was prepared. In this compound the angular methyl group at C_{10} is replaced by hydrogen. This structure resembles closely that of progesterone in that the characteristic side chain is left unchanged.

The 10-nor-progesterone was prepared by a series of chemical transformations starting with strophanthin.[1]

[1] Maximilian Ehrenstein, *Jour. Org. Chem.*, Vol. 9, No. 5, September, 1944.

The end product was a resinous substance. The ultraviolet absorption spectrum possesses the characteristics of an α, β-unsaturated ketone ($\lambda - max = 238.5$ mμ; $\varepsilon = 16560$). Though the substance is obviously pure as to its chemical structure, it is believed to

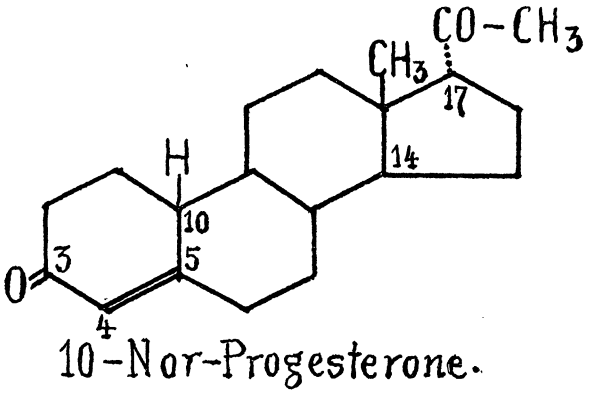

FIG. 1

represent a mixture of stereo-isomers. The extent to which carbon atoms 10, 14 and 17 may be involved in such stereo-isomerism is discussed elsewhere.[1]

The activity of this compound was tested in castrated, sexually mature rabbits according to the method of Corner and Allen.[2] The rabbits were mated and castrated about 18 hours later. Care was taken not to traumatize the tubes in order that the fertili eggs might pass normally into the uterus. The first rabbit received a total of 4.1 mg over a period of five days and at autopsy six normal blastocysts were found in the uterus. The endometrium showed full proliferation. In view of this result a second rabbit was given a total of 0.83 mg, but in this experiment no blastocysts were found in the uterus. The endometrium, however, showed complete proliferation. The failure to find blastocysts does not, of course, indicate that the endometrium was abnormal since in many similar experiments with pure progesterone blastocysts are frequently not found.

This new compound appears to be fully as active as progesterone, perhaps even more so, since the minimum amount of progesterone which produces full proliferation in the sexually mature rabbit is about 1.0 mg. All other compounds related to progesterone which have progestational activity are considerably less active than progesterone. These results indicate that the angular methyl group at C_{10} can be replaced with hydrogen without impairing the physiological activity.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR THE USE OF SOLID CARBON DIOXIDE AS A SOURCE OF CO_2 GAS

THE following apparatus has proved very useful in supplying small quantities of sterile carbon dioxide under low pressure to be used in adjusting the pH of tissue culture media in small flasks. The quantities of carbon dioxide needed for such pH adjustments often do not warrant the procuring of a reducing valve, with an adapter to fit the smallest size cylinders, such as would be required to keep the pressure within usable limits.

A 750 ml suction flask was fitted with a one-hole rubber stopper and a short piece of glass tubing, bent at right angles, inserted into the stopper. To this was attached a short piece of rubber tubing, about 3 inches long, and a small Hoffman screw clamp. A short length of rubber tubing was attached to the side arm of the flask and connected to a glass T on one leg of a simple open-end U-tube mercury manometer. The manometer was calibrated from 0 to 3 pounds. A flask, filled with non-absorbent cotton and sterilized by autoclaving, was connected to the other outlet of the T by a length of rubber tubing.

To use the apparatus, a few pieces of solid carbon dioxide, "dry ice," were placed in the flask, the stopper inserted and the Hoffman clamp tightened sufficiently to allow the pressure to build up to about one or one and one-half pounds. This pressure was maintained by tightening or loosening the screw clamp. Gas was allowed to evolve until all the atmospheric air had been displaced from the system.

With the apparatus now ready for use, the pressure could be controlled at will by means of the screw clamp. The apparatus safely maintained a pressure of 3 pounds for one-half hour. The rate of evolution of gas dropped off somewhat after about 10 minutes due to the cooling of the flask, but there was sufficient gas being evolved to keep the pressure at about one pound. This is usually ample for the needs considered in this paper.

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[1] G. W. Corner and W. M. Allen, *Am. Jour. Physiol.*, 88: 326, 1929.

[3] Aided by a grant from the Smith, Kline and French Laboratories in Philadelphia.