The significance of these results approximates the 93% level(11,12).

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Phenethylbiguanide and Triglyceride Synthesis.* (27919)

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The oral hypoglycemic agent, phenethylbiguanide (PEBG)‡ has been shown *in vitro* to increase the uptake of glucose by rat adipose tissue(1) and thus to have an effect similar to insulin. Unlike insulin, however, PEBG inhibits oxygen uptake by adipose tissue(2) and increases lactic acid production by rat diaphragm(3). PEBG, therefore, acts differently from insulin in certain respects, and the following experiments were done to ascertain its effects on triglyceride synthesis in isolated adipose tissue, a reaction shown to be increased by insulin(4).

Methods. Intact tissue. Sprague-Dawley rats, 200-300 g, were killed by a blow on the head and exsanguination. The epididymal fat pads were excised and portions, approximately 50-80 mg, were weighed and incubated in 3 ml Krebs-Ringer-Bicarbonate buffer, pH 7.4, containing 5 μM glucose, 2% crystalline bovine albumin, and 1-C^{14}-palmitic acid. Incubations were carried out for 3 hours in a Warburg apparatus under an atmosphere of 95% O_2-5% CO_2. Where oxygen uptakes were measured Krebs-Ringer-Phosphate buffer was used as the incubation medium and 100% O_2 as the atmosphere. PEBG was used in the concentrations noted in the figures.

Following incubation, the fat pads were removed, blotted and rinsed 3 times in 0.9% NaCl. They were homogenized with 10 ml of chloroform-methanol (2:1 volumes), filtered, and dried under nitrogen. Lipid fractionation was carried out as described below.

Homogenates. After removal from the rats, the epididymal fat pads were homogenized in KCl-Tris buffer, pH 7.4, using 200 mg tissue per ml of buffer. The homogenates were centrifuged at 800 $\times$ *g* for 20 minutes, and the middle layer, between the nuclear debris and the fat, was removed and filtered through cotton. Incubation flasks were then prepared in a manner similar to that of Steinberg, *et al.*(5) and held 2 ml of homogenate, 1 ml, KH_2PO_4 buffer, pH 7.4 containing 20 μM PO_4, 10 μM ATP, 2 μM $MgCl_2$, 10 μM α-glycerophosphate and 0.08 μc sodium 1-C^{14}-palmitate. Incubations were carried out for 90 minutes in room air at 37°C. PEBG was used in the concentrations noted in the figures.

Following incubation, 2 ml of medium was removed, acidified, and extracted twice with 7 ml aliquots of petroleum ether (b.p. 30-60°C). These were pooled and dried under nitrogen. Lipid fractionation was carried out as described below.

Fractionation of lipids. The dried extracts were taken up in hexane and placed on a sili-

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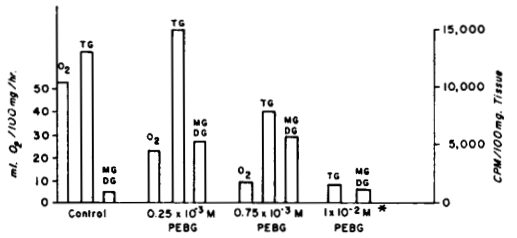

FIG. 1. PEBG decreased oxygen consumption of adipose tissue. The smaller dose levels increased incorporation of C^{14} from 1-C^{14}-palmitic acid into monoglycerides and diglycerides (MG, DG); larger doses decreased its incorporation into triglycerides (TG).

cic acid column and the lipids eluted according to the solvent systems of Barron and Hanahan(6). The triglycerides were separated from the free fatty acids by an alkali-ethanol wash(7). The triglycerides, free fatty acids and mono- and diglycerides were then transferred to individual counting vials and radioactivity was determined in a Packard Tri-Carb Liquid Scintillation Counter.

Results. Intact adipose tissue readily incorporates 1-C^{14}-palmitic acid from the medium into tissue glycerides, and as seen in Fig. 1, this appears largely in the triglycerides with only a small amount as mono- and diglycerides. When PEBG is added to the medium in a concentration of 0.25×10^{-3} molar, the total fatty acid incorporation is increased but this increase appears almost entirely in the mono- and diglyceride fraction. The respiration of the tissue is, however, depressed as reflected by the inhibition of oxygen uptake. When the concentration of PEBG is increased to 0.75×10^{-3} molar, the incorporation of medium 1-C^{14}-palmitic acid into tissue triglyceride is markedly suppressed but the incorporation of the fatty acid into mono- and diglycerides remains at a high level and the oxygen uptake of the tissue is further decreased. By adding PEBG to a concentration of 1×10^{-2} molar (Fig. 1), the incorporation of 1-C^{14}-palmitic acid into triglycerides is almost abolished while the previously elevated incorporation into mono- and diglycerides is repressed to control levels.

In the experiments utilizing homogenates of adipose tissue, PEBG at a concentration of 2×10^{-3} increases the incorporation of 1-C^{14}-palmitic acid into triglycerides (Table I) and this is significant at the 1% level. The effect on the mono- and diglycerides is insignificant.

Discussion. While *in vitro* both PEBG and insulin increase glucose uptake by adipose tissue of normal rats(1,8), and insulin increases the incorporation of palmitic acid into triglycerides(4), the present experiments show that PEBG does not cause this increased incorporation into triglyceride although it does cause an increase in the incorporation of labeled palmitic acid into mono- and diglycerides.

The synthesis of triglycerides is thought to occur by the following steps(5):

I Fatty acid + CoA $\xrightarrow{\text{ATP}}$ acyl CoA.

II 2 acyl CoA + α-glycerophosphate ⟶ phosphatidic acid.

III Phosphatidic acid ⟶ diglyceride.

IV Acyl CoA + diglyceride ⟶ triglyceride.

Thus it would seem that an increase in radioactivity of the diglycerides should be reflected by an increase in radioactivity in the triglycerides. When PEBG is added to the medium, however, it causes an increase in mono- and diglyceride radioactivity of the isolated fat pad, but no change, or a marked decrease in triglyceride activity, depending on its concentration. This implies that some block in the conversion of diglycerides to triglycerides was brought about by PEBG. When PEBG is added to homogenates fortified with ATP, however, this block is no longer present, and there is an increase in triglyceride radioactivity. Thus PEBG does not seem to interfere with any enzymes con-

TABLE I. Effect of PEBG Recovered in Triglyceride and in Monoglyceride-Diglyceride Fractions of Homogenate, Based on Radioactivity Measurements from Added 1-C^{14}-Palmitate.

		cpm (± S.E.M.) recovered as:	
	No. of exp	Triglycerides	Monoglycerides & diglycerides
Control	5	24,050 (±870)	2,340 (±200)
		$p < 0.01$	$p > 10$
PEBG, 2×10^{-3} M	5	32,000 (±1,480)	2,780 (±480)

cerned directly with esterification of diglyceride.

We feel that the following hypothesis is in keeping with the known blocking action of PEBG on oxidative phosphorylation(9). By blocking oxidative phosphorylation in intact tissue, PEBG decreases the amount of available ATP. Since step I in the formation of glycerides involves ATP, a decrease in ATP will curtail this reaction and less acyl CoA, necessary for steps II and IV, will be produced. The limited amount of acyl CoA produced is competed for by α-glycerophosphate (II) and diglyceride (IV) and since the former is in greater abundance, it has a much greater chance to react with acyl CoA. Accordingly, the α-glycerophosphate is converted to diglyceride, but the latter never accumulates sufficiently to compete successfully for the limited amount of acyl CoA and thus remains as diglyceride instead of being rapidly converted to triglyceride. In the homogenates, however, where excess ATP is supplied, the formation of acyl CoA is not limited. With the interference with oxidative phosphorylation and secondarily with the Kreb's cycle there is a relative accumulation of the 3-carbon fragment lactate(10). It is possible that other 3-carbon fragments also accumulate and there would be more α-glycerophosphate to be converted to triglycerides; thus an increased amount of the latter is formed.

Summary. The effect of PEBG on triglyceride synthesis has been studied *in vitro*. In intact tissue PEBG interferes with the conversion of diglyceride to triglyceride. In homogenates, PEBG increases the incorporation of labeled fatty acid into triglyceride. It thus differs from insulin in its effect on triglyceride synthesis. An explanation for this is presented.

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Action of Deoxycorticosterone Acetate* on Glycogen. II. Relationship of Muscle Glycogen Formation to Potassium Metabolism.† (27920)

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A recent report from this laboratory(1) indicated that inhibition of liver glycogen formation after repeated daily administration of desoxycorticosterone acetate (DOCA) to intact rats was secondary to action of the steroid on potassium metabolism.

It has been reported(2) that the mechanism of glycogen formation in skeletal muscle might not be the same as that in liver. It was therefore of interest to determine if glycogen formation in skeletal muscle of intact rats was also inhibited by DOCA, and if so,

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