and malignant trophoblast of placental and testicular origin.

Summary. This report describes the immunobiological characterization of an antiserum to commercial human chorionic gonadotropin. This antiserum identified human chorionic gonadotropin of benign or malignant trophoblastic origin in serum or urine and neutralized its biological activity.

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Effect of 2,4-dinitrophenol on Free Fatty Acid Uptake by Skeletal Muscle.* (26854)

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It was reported(1) that in the anesthetized dog during direct electrical stimulation the muscles of the hind leg showed a consistent uptake of free fatty acids (FFA) on the stimulated side. A metabolic change very similar to that induced by muscular contraction can be produced by dinitrophenol (DNP) poisoning. Although the detailed mechanism of action of this "uncoupler" on the electron transport system is not yet entirely understood and the question whether it inhibits the formation of energy rich P bonds or enhances the hydrolytic cleavage of the terminal phosphate of ATP is a matter of discussion(2,3,4), it can be safely stated that DNP induces an imbalance between formation and breakdown of the high energy P compounds of the cell. The result of this imbalance is a dramatic rise of the metabolic rate.

Experiments carried out with a method similar to that used in this study had shown that in the anesthetized dog DNP increased blood flow, oxygen uptake and lactic acid production of the muscle 5-9-fold, glucose uptake was increased about 2-fold, and the muscle glycogen decreased about 40% in 60 minutes. A considerable loss of inorganic phosphate and a concomitant rise of plasma inorganic P level were also recorded(5,6).

The muscle creatine phosphate content dropped 59% while ATP content decreased only 13%(5,6). As far as the metabolism of muscle is concerned, DNP poisoning can be considered as exhausting work lasting for about 1-2 hours, the only difference being that the liberated energy is wasted in the form of heat without performing work.

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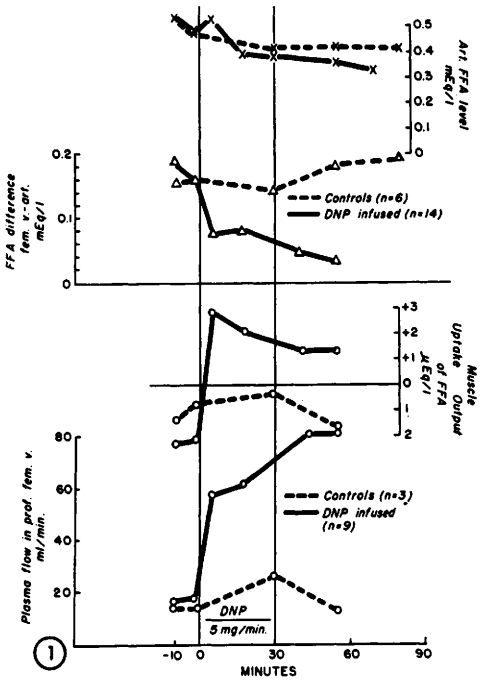
Earlier investigations(7) suggested that DNP may increase oxidation of fat in the unanesthetized intact animal. DNP decreased the RQ of rats from 0.93 to 0.78. It was also shown in dogs that the increase in O_2 uptake induced by DNP was considerably higher than would have been required for oxidation of carbohydrate which disappeared from the body. Since N excretion was not increased, the conclusion was drawn that DNP accelerated oxidation of fat. Considering these findings it was of interest to study the uptake of FFA by the muscle under the effect of DNP.

Methods. Adult mongrel dogs (body weight: 15-25 kg) of both sexes were studied in the post-absorptive state under sodium pentobarbital anesthesia (30 mg/kg i.v.). Blood flow was determined according to the method of Issekutz Jr. *et al.*(8) in the right profunda femoris veins which drain predominantly muscular areas. Plasma flow was calculated from simultaneous determinations of blood flow and hematocrit.

In 9 animals estimated hepatic plasma flow (EHPF) was determined by the BSP dilution method of Bradley *et al.*(9) measuring portal and hepatic venous blood BSP levels (10).

Blood samples were taken simultaneously from the following vessels: right femoral artery, and right profunda femoris, left femoral, portal and hepatic veins. The blood samples were placed in chilled oxalated tubes, centrifuged, separated, and precipitated in the cold. FFA was determined in duplicate analyses by the method of Dole(11).

DNP solutions for infusion were prepared by dissolving the commercial preparation in water made alkaline by addition of a few drops of 1 N NaOH. In control experiments infusion of a salt solution with pH adjusted to that of the DNP mixture was used. Both DNP and BSP infusions were given with Harvard constant rate infusion pumps. The dose of DNP was adjusted so that each animal received 2 ml/min of a solution containing 2.5 mg/ml for 30 minutes. Intra-arterial infusions were made into the femoral artery just distal to the profunda femoris branch, so that the drug would be carried directly to the area supplied by the latter vessel.



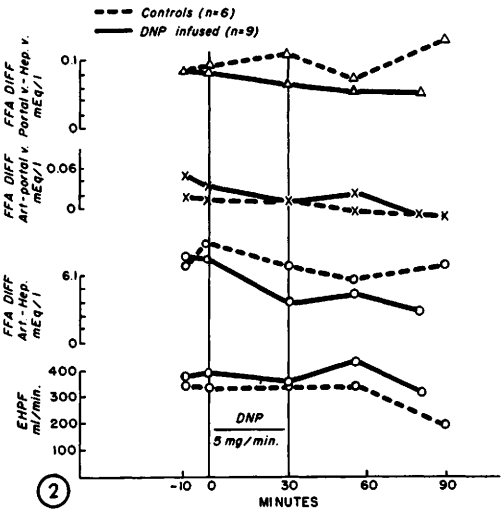


FIG. 1. Effect of DNP on plasma flow and FFA uptake in profunda femoris veins.
FIG. 2. Effect of DNP on hepatic uptake of FFA.

Results. Fig. 1 shows the effect of DNP on plasma flow in the profunda femoris vein, FFA output and uptake by this area, arteriovenous (femoral vein) FFA difference in the other leg and arterial FFA level. The curves represent average values obtained in 9 animals. Plasma flow was increased by intra-

arterial infusion of DNP to 4-5 times the initial value. At the same time the FFA output of the area which was found in all dogs prior to infusion, was changed to a definite FFA uptake in 8 of 9 experiments. The mean difference between pre-infusion FFA output and average uptake measured in the first 60 minutes after the start of DNP infusion was 3.83 μEq/min with a standard error of $\pm$0.9 μEq/min. This difference was significant at the $p<0.01$ level.

Blood of the femoral vein always contained more FFA than arterial blood. This difference decreased after DNP administration to about one-fifth the original value. The mean difference and its standard error was 0.128 $\pm$0.026 mEq/l. This change is significant at the $p<0.001$ level.

The slow decrease of arterial level from 0.48 mEq/l to 0.33 mEq/l shown by the average curve is not significant. Of 14 dogs it was decreased in 6, increased in 5 and unchanged in 3. In 9 experiments hepatic plasma flow was also estimated (EHPF) and FFA differences (mEq/l were measured across the splanchnic area (artery-portal vein) and across the hepatic portal system (Fig. 2). No striking change was observed during DNP poisoning. The decrease of arterio-hepatic FFA difference shown on the average curve is not significant (average decrease: 0.061 $\pm$ 0.031 mEq/l; $0.05<p<.1$). Since the possibility could not be ruled out that DNP poisoning limited the validity of the indirect estimation of hepatic plasma flow, a calculation of hepatic FFA uptake does not seem warranted.

Discussion. Blood in the profunda femoris veins carries more FFA than arterial blood, meaning that FFA uptake by the skeletal muscle of the anesthetized dog at rest is either zero or so low that the continuous release of FFA from the adipose tissue present in this area can mask it entirely. However, when the energy requirement of the muscle increases and turnover rate of the tricarboxylic acid cycle in turn increases many fold (either due to electrical stimulation(1) or to administration of DNP), then FFA uptake by muscle becomes measurable and the blood in the profunda femoris veins contains less FFA than the arterial blood.

After administration of DNP the femoral vein—arterial FFA difference decreases strikingly. Concentration in the former, however, remains higher than in the arterial blood. Two factors may contribute to this effect: (a) increased blood flow and (b) increased FFA uptake of the muscles drained by the femoral vein.

The alternative explanation that FFA output by the adipose tissue of the leg was decreased seems unlikely because (a) hepatic uptake of FFA did not seem to show a significant decrease; (b) FFA uptake by the muscle was elevated; and (c) considering the small pool size of the albumin bound FFA, a decreased release would be expected to induce a sharp drop of arterial FFA level. This was not the case.

Summary. Uptake of free fatty acids by skeletal muscle was determined by simultaneously measuring blood flow through profunda femoris veins, and arteriovenous fatty acid differences in anesthetized dogs. Administration of 2.5 mg/ml of DNP for 30 minutes caused elevation of blood flow and uptake of free fatty acids by the skeletal muscle.

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