The results of the preliminary test indicate that the acetic acid, when alone or in combination, offers promise as a safe and effective tomato seed soak for the control of bacterial canker. Further studies to establish limits of concentration and effective schedules for the treatment are in progress.

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VITAMIN B_1 AND THE SYNTHESIS OF FAT FROM CARBOHYDRATE

IN recent years it has become clear that vitamin B_1 is concerned with carbohydrate metabolism. Professor R. A. Peters[1] has ably reviewed the evidence which substantiates this belief, and he has provided a theory for the action of the vitamin in preventing or curing polyneuritis. As stated by Peters, "This vitamin is a catalyst used by the tissue at some stage in the combustion of carbohydrate. Defect in this stage within the central nervous system will lead readily to convulsions."

The theory of the action of vitamin B_1 as defined to date is, then, that carbohydrate metabolism proceeds to the pyruvic acid stage but is there halted in the absence of vitamin B_1. In the presence of the vitamin the pyruvic acid is oxidized and energy production from carbohydrate is normal in amount. Emphasis has been placed upon the action of vitamin B_1 as a catalyst necessary for the combustion of carbohydrates. There is no doubt that the vitamin permits the oxidation of pyruvic acid by brain tissue *in vitro* and the explanation may be completely satisfactory for that tissue. More recent evidence has indicated that the vitamin has as a principal function in the body generally the synthesis of fat from carbohydrate.

Whipple and Church[2] have shown that the main factor in the weight increases due to vitamin B_1 in rats is the laying down of fat, and, in their experiments, the only possible source of this fat was the dietary carbohydrate. Further evidence was provided by them[3] in measurements of respiratory quotients that carbohydrate is transformed into fat under the influence of vitamin B_1. The writer has confirmed[4] the production of fat from carbohydrate in the presence of the vitamin.

At this stage an hypothesis regarding the action of vitamin B_1 might be advanced, based, it is true, upon incomplete evidence. Whether or not the vitamin is supplied, it has been generally accepted that carbohydrate metabolism proceeds to the pyruvic acid stage. In the absence of the vitamin pyruvic acid accumulates as has been shown in pigeons, rats and in human subjects by a number of workers. In the presence of the vitamin fat is synthesized, presumably with pyruvic acid as an intermediary stage between carbohydrate and fat, although this is as yet unproven. It has long been felt that this possibility exists and recently Krebs and Johnson have shown[5] that hydroxy butyric acid can be formed from pyruvic acid by tissues.

This hypothesis, which attempts to correlate various pieces of published evidence, is that vitamin B_1 is necessary for the synthesis of fat from carbohydrate. It does not weaken the belief that vitamin B_1 is concerned with carbohydrate metabolism but alters the conception of the vitamin being a factor in energy production from carbohydrate to a broader view of carbohydrate utilization. An explanation is suggested for the disappearance of pyruvic acid when vitamin B_1 is supplied to avitaminous birds or animals and for the laying down of fat under such conditions. Furthermore, the action of dietary fats in sparing vitamin B_1 might be through provision of the body with necessary fat which on diets poorer in fat would be synthesized by the animal from carbohydrate with the help of vitamin B_1.

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THE EXPERIMENTAL PRODUCTION OF INTERSEXUALITY IN THE FEMALE RAT WITH TESTOSTERONE

IN a previous report[1] the observations of Hain[2] on the production of hypospadias in the female offspring of the rat by the injection of the mother with estrone,[3] before or immediately after birth, have been confirmed. We have now found that estradiol[3] injected into the mother (2.0–3.0 mg) antepartum or into the *new-born* female (0.2–0.4 mg) also produces hypospadias. The male offspring were apparently not influenced. On the basis of embryological facts, it was suggested[1] that the hypospadias was due to an hypotrophic defect. This immediately suggested the idea that testosterone[3] when given to the pregnant rat might (a) cause hypospadias in the male offspring, or (b) produce an arrest of the development of the vagina in the female, or (c) produce intersexuality (free-martin) in the female. These latter two possibilities have now been shown to be true.

1 R. A. Peters, *Lancet*, 230: 1161, 1936.
2 D. V. Whipple and C. F. Church, *Proc. Amer. Soc. Biol. Chem.*, 30: cvii, 1936.
3 Whipple and Church, *ibid.*, 31: ciii, 1937.
4 E. W. McHenry, *Jour. Physiol.*, 89: 287, 1937.
5 H. A. Krebs and W. A. Johnson, *Biochem. Jour.*, 31: 645, 1937.
1 R. R. Greene, *Proceedings Soc. Exp. Biol. and Med.*, 36: 503, 1937.
2 A. M. Hain, *Quart. Jour. Exp. Physiol.*, 25: 131, 303, 1935; *ibid.*, 26: 290, 293, 1936.
3 We desire to thank Dr. Oliver Kamm, of Parke, Davis and Company for the estrone, and Dr. E. Schwenk, of the Schering Corporation, for the estradiol and testosterone used in this work.

Testosterone and testosterone propionate in varying doses have been administered to rats at varying periods of pregnancy. A large percentage of resorptions or still births have resulted. To date, however, seven litters have been obtained. In these litters there were twenty-seven normal males and nineteen females with varying degrees of intersexuality. Three of these litters are now completely mature (67 to 70 days, with weight 150 to 170 gms) and display no evidence of further "feminization."

A rudimentary but patent vagina is present in the female adult offspring of one litter, whose mother received a small dose of testosterone late in pregnancy (3.3 mgm on nineteenth day). In all other animals the pelvic vagina is absent (distal portion). In all animals dissected to date, mature or new born, attached to the proximal urethra at the base of the bladder, are paired glandular structures that histologically resemble prostate. In addition other glandular tissue that histologically resembles seminal vesicles is found.

In adult animals that have been killed apparently normal ovaries (corpora lutea present in one case) have been found with seemingly normal oviducts and uteri that end in a dilated, bulbous structure that represents the proximal portion of the vagina.

Other animals, whose mothers received the male sex hormone earlier in pregnancy, show varying degrees of inhibition of Mullerian duct, and stimulation of Wolffian duct derivatives. In one animal, whose mother received 2.5 mgm testosterone propionate on the twelfth day of pregnancy, the oviducts are seemingly absent and the uteri are represented by a very rudimentary structure immediately posterior to the bladder. Prostate, seminal vesicle and a rudimentary vas deferens are present.

The external genitalia of affected animals vary. The offspring of mothers receiving the male sex hormone late in pregnancy have a crescentic fold of skin that represents the vaginal orifice surrounding the caudal base of an organ that resembles a "hypospadic" clitoris. With larger doses given earlier in pregnancy the organ resembles a penis, but is smaller than the penis of a litter brother.

It is rational to expect that male sex hormone may influence the development of the primordia of the female genitalia as late as the twelfth to fourteenth day of gestation, because the early development of the rat embryo is very slow, *e.g.*, the mesodermal layer does not appear until the ninth day.[4] It is known that if in cattle[5] or pigs[6] the circulation of twin male and female embryos is interconnected, the female becomes modified in the male direction. Lillie[5,6] postulates that this is due to the effect of a male sex hormone upon the anlage of the genitalia of the female embryo. Further, a report by Dantchakoff,[7] which was found during the preparation of this communication, shows that on injecting testosterone into the amniotic sack of embryo guinea pigs, agenesis of the vagina and other evidences of intersexuality result. Thus our results on the rat are quite analogous to those on the guinea pig.

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CLOT PREVENTION IN BLOOD STUDIES IN ANIMALS

THE blood of laboratory animals, such as rabbits and rats, clots very rapidly, making blood counts rather difficult, especially when large numbers of such counts have to be made daily and over a long period of time. Blood films from such animals are likewise not always satisfactory, owing to the fact that the cells form small clusters that cover a good portion of the slide, at times including such of the morphological elements as are to be particularly studied. The subsequent cleaning of the pipettes is difficult and time-consuming.

To avoid these handicaps the writer has, for the past several years, been using a simple method which apparently overcomes all such difficulties. With this method the blood films are always satisfactory and legible and always successfully stained vitally, the counting of the blood cells is easily carried out and the cleaning of the pipettes offers no problem. This method is as follows:

When rabbits are used the ear is rubbed gently with a piece of gauze, previously dipped into a saturated solution of sodium citrate. The excess citrate solution is wiped away, leaving the surface very slightly moist. This causes the veins of the ear to dilate greatly, such dilations being greater than when heat or acetone had been applied to the animal's ear. Puncturing of a vein over this area permits the blood to flow freely, and collection in a counting pipette or on a slide or cover-slip for smearing offers no difficulty, since the minute particles of sodium citrate that remain on the skin and the hairs prevent coagulation.

When rats are used the end of the tail of the animal

[4] G. C. Huber, *Jour. Morph.*, 25: 247, 1915.
[5] F. R. Lillie, *Jour. Exp. Zool.*, 23: 371, 1917.
[6] W. Hughes, *Anat. Rec.*, 41: 213, 1928–29.
[7] V. Dantchakoff, *Compt. rend. Soc. d. Biol.*, 174: 516 (March), 1937.